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Models of PD

Principal Investigator: ACTON, PAUL D
Grant Number: 1R01NS048315-01
Title: Imaging stem cell implants in neurodegenerative disease

Abstract: Despite considerable effort, there is still no reliable way to either prevent or rescue dopamine (DA) neurons from the progressive degeneration that occurs during aging or in Parkinson's Disease (PD). Since clinical diagnosis almost always occurs after the vast majority of DA neurons have been destroyed, researchers continue to work to develop ways in which to replace lost tissue with transplanted cells capable of dopaminergic function. Our goal is l to study purified populations of engineered stem, progenitor or fetal DA neurons after transplantation into the Parkinsonian rat. A major limitation of these approaches is the inability to monitor the progression, if any, of the grafted cells without highly invasive tissue biopsy, which invariably results in the death of the animal. The vast numbers of animals required for these studies could be reduced significantly (with the concomitant reduction in costs) if each animal could be studied non-invasively and repeatedly. In addition, measurements made of the DA neuronal regeneration in a rat model ex vivo are not translatable to humans. Molecular imaging, using PET and SPECT, of animal models of PD, and other neurodegenerative diseases, enables the study of the in vivo neurochemical basis of the disorder. In this proposal we aim to perform quantitative imaging of dopaminergic neurons in vivo in longitudinal studies of the same animals over an extended period of time after stem cell implantation. We aim to validate quantitative models of dopaminergic function in rats, using ultra-high resolution] PET and SPECT. [18F]DOPA imaging will be used with PET to monitor striatal dopa decarboxylase activity, while [99mTc]TRODAT-1 and SPECT will be used to measure dopamine transporter (DAT) availability directly. These will be validated against established post mortem methods, such as GFP reporter gene expression and immunocytochemistry. Longitudinal imaging studies of rats, following stem cell implantation, will enable the visualization of the regeneration of DA neurons over an extended period of time. Once the imaging techniques have been fully validated, they will be applied to a variety of stem cell implant models, and correlated with behavioral studies. This non-invasive approach will enable the best combination of cell types and growth factors to be established without sacrificing the animals. The ultimate goal of this study is to develop methods which will enable the monitoring in vivo of DA neuron replacement treatments in Parkinson's and other neurodegenerative diseases. This will provide vital information in the animal model of PD, allow us to longitudinally follow DA neuron regeneration, and, most importantly, will be translatable to

Principal Investigator: ANDERSON, MARJORIE
Grant Number: 5R01NS044565-03
Title: Deep Brain Stimulation in Parkinson's Models

Abstract: Although high-frequency deep brain stimulation (HF-DBS) in the globus pallidus or subthalamic nucleus has become a common technique used to treat drug-resistant symptoms of Parkinson's disease, the mechanisms by which HF-DBS exerts its effects are unknown. In the proposed studies, the ability of chronic administration of the insecticide rotenone, to produce an animal model of Parkinson's disease will first be tested in monkeys. Using PET imaging now available in the University of Washington Regional Primate Research Center, changes in dopamine innervation after administration of rotenone will be measured using a marker of the monoamine vescicular transporter that is present in dopaminergic nerve terminals. These changes will then be correlated, over time, with changes in behavior and with electrophysiological changes in the rate and pattern of discharge of neurons in basal ganglia-receiving areas of the thalamus. This model will then be used to couple the electrophysiological effects of HF-DBS, which can be recorded from basal ganglia-receiving neurons of the thalamus, to the stimulation-induced changes in regional metabolism in the cortex and thalamus. PET imaging with the metabolic marker, [8-F] flurodeoxyglucose (FDG), will be used to measure metabolism. This technique has generally shown a relative hypermetabolism in the globus pallidus and thalamus of humans with Parkinson's disease and a relative hypometabolism in areas of the frontal cortex. Changes reported to be induced by HF-DBS have been mixed however. The combination of electrophysiology and metabolic imaging will allow us to address some of the discrepancies from the human literature. Special attention will be paid to the development of abnormal patterns of bursting behavior in the thalamus of monkeys treated with rotenone, as well as the effect of HF-DBS on burst behavior. This will test the hypothesis that some of the symptomatology of Parkinson's disease, and its relief using HF-DBS, is a consequence of abnormal patterns of activity in basal ganglia-thalamic-cortical circuits.-

Principal Investigator: Bakay, Roy A
Grant Number: 1R01NS046612-01A1
Title: Stem Cells in CNS Transplantation

Abstract: Stem cells offer tremendous promise for the future of transplantation. We propose examining embryonic stem cells (ESC) in monkey allografts. We will compare dopaminergic enriched ESC to fetal mesencephalic (FM) neurons in their ability to survive, innervate, and restore lost function in the best animal model of PD, the MPTP treated monkey. The primate is essential for this study to test the hypothesis that replacement strategy must completely reinnervate the very large volume of the monkey striatum. Recently clinical trials have indicated that dopaminergic (DAergic) replacement with FM neurons can cause severe debilitating dyskinesia. It is then imperative to have a clear understanding of how a DAergic enriched ESC replacement strategy affects l-dopa-induced dyskinesia (LID). In this regard, we will also compare the effects of FM transplants and DAergic enriched ESC upon the dyskinesia profile of MPTP monkeys. The potential to induce or diminish dyskinesia will be tested with the best model of dyskinesia (primate LID model). The key problem of parkinsonian transplantation with fetal or stem cells grafts is the incomplete reinnervation of host striatum. Like the FM transplant patients, focal areas of relative hyperdopaminergic activity should render these monkeys highly susceptible to LIDs. Thus to optimize reinnervation and functional recovery while minimizing the potential for dyskinesia, we will also treat DAergic enriched ESC with glial cell line-derived neurotrophic factor (GDNF) delivered via a lentiviral vector. The lenti-viral vector is critical to this hypothesis because of the proven ability to transfect the entire striatum and act not as a point source but as a volume source to stimulate reinnervation. Intraparenchymal GDNF released diffusely throughout the entire striatum should act as a developmental cue for these immature cells to extend DAergic processes throughout the striatum as well as provide neuronal rescue for dopaminergic neurons in the pars compacta of the substantia nigra. Sufficient subjects and multiple controls are included to insure proper interpretation of the data. The present series of experiments serves to provide the essential preclinical data needed to help determine the utility of nonhuman dopaminergic enriched stem cells. -

Principal Investigator: BAUDRY, MICHEL
Grant Number: 1R01NS048521-01A1
Title: Calpain inhibitors in models of Parkinson's disease

Abstract: Parkinson' s disease is a neurodegenerative disease that specifically affects dopaminergic neurons in the substantia nigra. Although several hypotheses have been proposed to account for the specificity of the neurodegenerative features of the disease, the exact cause of the disease remains to be elucidated. Significant advances in our understanding of the possible causes of the disease were provided by the serendipitous discovery that a neurotoxin, 1-methyl-4-phenyl-1,2,3,6- tetrahydropyridine (MPTP), elicits a pattern of neurodegenerative features in humans and experimental animals identical to that seen in patients with Parkinson' s disease. A potential target to prevent neurodegeneration in Parkinson' s disease is the calcium-dependent protease calpain. Calpain levels are elevated in post-mortem substantia nigra of patients with Parkinson' s disease, MPP+ neurotoxicity in granule cell cultures is associated with calpain activation and blocked by calpain inhibitors, and calpain has been implicated in several neurodegenerative diseases. We have recently obtained a series of novel and potent calpain inhibitors and have demonstrated their potency in preventing NMDA-induced calpain activation in cultured hippocampal slices. The current proposal is aimed at testing the hypothesis that calpain activation plays a critical role in animal models of PD and that calpain inhibitors are neuroprotective in these models. We will first determine the potency and efficacy of calpain inhibitors to prevent MPTP toxicity in cultured slices from rat mesencephalon. We will then use structure activity relationship in conjunction with additional assays to identify the best inhibitors to be tested in in vivo models. Finally, we will test the hypothesis that calpain is activated and that calpain inhibitors are neuroprotective against MPTP-mediated neurotoxicity and behavioral impairments in vivo in C57Bl/6 mice, and against rotenone-mediated neurotoxicity in rats. Conversion of the pro-apoptotic factor Bid to its active, truncated form tBid will be tested as part of the mechanisms by which calpain activation induces cell death. These studies will test the hypothesis that calpain inhibitors might prevent neurodegeneration not only in Parkinson' s disease but also in a variety of conditions resulting from exposure to environmental toxins. Finally, because calpain has also been implicated in the mechanisms underlying Amyotrophic Lateral Sclerosis (ALS), our proposal could lead to significant advances in the treatment of this neurodegenerative disease as well. -

Principal Investigator: BENNETT, JAMES P
Grant Number: 5R01NS039005-05
Title: OXIDATIVE STRESS IN PARKINSON'S DISEASE

Abstract: Idiopathic Parkinson's Disease (PD) is a major neurodegenerative disease affecting at least 1 million Americans, and the cellular cause of PD is not yet known with certainty. This proposal will explore further the central hypothesis that defects in mitochondrial electron transport chain (ETC) function are a major contributor to premature cell death in PD and will address four Specific Aims 1) define the pathophysiology of mitochondrial transition pore function, and how regulation of membrane potential and intracellular calcium signaling are altered in PD; 2) determine mechanisms of Bcl protein regulation in PD cybrids, and whether transfection with Bcl-overexpression vectors alters mitochondrial function and improves survival; 3) further define the interactions among MAPKinase signaling pathways and NFkappaBeta transcription factor in PD; and 4) characterize mitochondrial transition pore complexes isolated from human postmortem PD brain and compare their function to those isolated from control brain. This project will make use of state-of-the- art intracellular ion imaging technology, RT-PCR techniques, gene transfection strategies, and will develop cell-free systems to examine several inter-related hypotheses. Behind all of these laboratory experiments is a therapeutic imperative, which will be explored in cell and cell-free models. Because new data presented in this application supports the hypothesis of systemically increased oxidative stress in PD patients, exploring these events in an established cell model is even more compelling. This proposal will also compare findings in PD cybrids with those in SY5Y cells exposed to chronic rotenone treatment, a pharmacological cell-based model of complex I loss. Ultimately, the results from this proposal will establish the central importance of genetically acquired mitochondrial ETC dysfunction as an etiologic factor in sporadic PD. Paradigms for evaluating neuroprotective therapies will also be developed to allow targeted approaches to correcting consequences of increased oxidative stress in cells. -

Principal Investigator: BING, GUOYING
Grant Number: 5R01NS044157-02
Title: Cox-2 deficient mice are resistant to MPTP neurotoxicity

Abstract: Parkinson's disease (PD) is a movement disorder characterized by the progressive loss of dopamine-containing neurons in the substantia nigra pars compacta (SNpc). Loss of SNpc dopaminergic neurons results in the depletion of striatal dopamine levels and produces symptoms such as tremor, muscle rigidity, and bradykinesia. The etiology of PD is unknown, but chronic inflammatory processes, microglial activation, and oxidative stress are thought to play prominent roles in the degeneration of dopaminergic neurons in the SNpc. Microglia are thought to contribute to neurodegeneration by releasing cytotoxic agents such as pro-inflammatory cytokines and reactive oxygen species that increase inflammation and oxidative stress. N-Methyl-4-phenyl-1,2,3,6,-tetrahydropyridine (MPTP) is a neurotoxin found to mimic many of the features of PD in animal models, including loss of dopaminergic neurons in SNpc and activation of microglia. Recent observations indicate that cyclooxygenase-2 (COX-2) deficiency in mice reduces the susceptibility of SNpc dopaminergic neurons to MPTP toxicity and diminishes MPTP-induced microglial activation. The purpose of this study is to test the hypothesis that COX-2-regulated inflammatory processes exacerbate MPTP neurotoxicity by activating microglia and increasing oxidative stress that contributes to the degeneration of dopaminergic neurons in the SNpc. To test this hypothesis, mice deficient in the COX-2 gene will be treated with MPTP to determine the role of COX-2 in MPTP-induced neurodegeneration. Furthermore, wild-type mice will be administered exogenous COX-2 inhibitors prior to MPTP treatment to evaluate the protective effects of COX-2 inhibitors against MPTP neurotoxicity. Following these experiments, dopaminergic neuron survival, microglial activation, striatal dopamine levels, and functional recovery will be assessed. In addition, protein modification, generation of reactive oxygen species, expression of inflammatory cytokines and apoptosis-related genes, and activation of specific signaling molecules will be evaluated to determine the molecular mechanisms by which COX-2 exacerbates MPTP neurotoxicity. The goals of this study are to elucidate the changes in inflammatory processing affected by COX-2 deficiency, to explore the etiology and molecular mechanisms underlying Parkinsonian symptoms in the experimental MPTP model, and to develop novel therapeutic treatments for PD and other neurodegenerative diseases.-

Principal Investigator: Bohn, Martha C
Grant Number: 5R01NS031957-08
Title: GENE THERAPHY FOR PARKINSON'S DISEASE

Abstract: The long-term goal of this project is to develop novel gene therapies for neurodegenerative diseases. In the previous support period, we focused on adenoviral (Ad) vectors to deliver the gene encoding GDNF (glial cell line-derived neurotrophic factor). Ad-GDNF injected into either the substantia nigra or striatum of a progressive degeneration model of Parkinson's disease protected dopaminergic (DA) neurons against cell death induced by the neurotoxin 6-OHDA. Ad-GDNF injected into the striatum also prevented the acquisition of behaviors and molecular changes that occurred in DA deficient young and aged rats. This proposal focuses on the hypothesis that anti-apoptotic gene delivery will also protect DA neurons in vitro and in vivo and have a synergistic effect with delivery of neurotrophic factor genes. Viral vectors harboring genes that block specific apoptotic death pathways, including XIAP, a dominant-negative caspase-9, bcl-2 and bclxl will be studied for effects on survival and function of DA neurons either alone or in combination with neurotrophic factors, GDNF or neurturin. Genes will be delivered to DA neurons in culture and in rat brain using helper free HSV:AAV hybrid amplicon vectors. These vectors will incorporate bidirectional expression cassettes that drive both the therapeutic gene and the cellular marker gene, green fluorescent protein, to permit specific evaluation of transduced cells. Expression will be controlled using the tetracycline responsive element such that transgene expression is "on" in the presence of tetracycline activator (TA) and in the absence of doxcycline (Dox). Vectors will be made in which TA is driven by a viral promoter of the DA cellular promoter, tyrosine hydroxylase (TH). Effects of the 'therapeutic' genes will be studied using non-neuronal cells, the DA cell line, MN9D, and primary fetal DA neurons treated with the neurotoxins, MPP+ or 6-OHDA or other cellular insults. In vivo effects of therapeutic genes will be studied in: 1) rats that have received grafts of fetal DA neurons, and 2) rats that have received a progressive 6-OHDA lesion of the nigrostriatal projection. Reversibility of effects will be studied by administration of Dox. Effects on DA neurons will be evaluated using quantitative morphometric and molecular techniques and behavioral evaluations. This project also aims to continue its evaluation of new generation viral vectors, including E2b deleted Ad, totally gutted Ad, and HSV:AAV amplicon, for stability and levels of expression in the nigrostriatal system. The studies involve collaborations among investigators at Children's Memorial Hospital and Northwestern Univ. Med. School and are relevant to the development of novel therapies for neurodegenerative diseases and injuries to the CNS. -

Principal Investigator: BONINI, NANCY M
Grant Number: 5R01NS043578-02
Title: Molecular Genetic Analysis of Neurodegeneration

Abstract: Many human neurodegenerative diseases are poorly understood as well as untreatable, including Parkinson's, Alzheimer's and Huntington's diseases. For some familial forms of these diseases, mutations in specific genes products associated with disease are known, allowing the possibility to model the disease in simple systems in order to address mechanisms of degeneration and to pioneer novel treatments. Toward this end, we applied a new approach to the problem of polyglutamine-induced neurodegeneration by developing a model for this class of human disease in the fruit fly Drosophila melanogaster. These experiments demonstrated that fundamental molecular mechanisms of polyglutamine-induced neurodegeneration are conserved in Drosophila, such that Drosophila genetics can be applied to investigate these human diseases in order to address mechanisms of degeneration and define new means of treatment. Using this model, we have shown that the molecular chaperones, which are highly conserved proteins, are potent modulators of neurodegeneration in vivo. We now propose to apply the powerful molecular genetics of Drosophila in genetic screens to uncover additional modulators of neurodegeneration. The advantage of genetic screens is that they provide the ability to define genes that can influence and modulate pathogenesis without requiring previous knowledge of the mechanisms involved. The specific aims are to define novel modulators of neurodegeneration in mis-expression and loss-of-function genetic screens, and to molecularly define and biologically characterize these modifiers in order to address their molecular and biological modes of action. By applying the power of Drosophila molecular genetics to address conserved features of polyglutamine-induced degeneration, these studies provide the foundation for new approaches to cures and treatments for human neurodegenerative disease. -

Principal Investigator: Botas, Juan
Grant Number: 5R01NS042179-04
Title: Neurodegeneration with Drosophila

Abstract: The ultimate goal of this project is to gain insight into polyglutamine-induced neurodegeneration by identifying genes, pathways and molecular mechanisms involved in the pathogenesis of spinocerebellar ataxia type 1 (SCA1). A Drosophila model of SCA1 was created by generating flies that express either normal or expanded human SCA1 transgenes. This fly model recapitulates the cellular phenotypes observed in SCA1 patients including the formation of nuclear inclusions (NI) and progressive neuronal degeneration. Capitalizing on the power of Drosophila genetics, two large genetic screens were designed to identify genes that modify a SCA1 neurodegenerative phenotype in the eye. The first screen yielded modifiers of the SCA1 phenotype when gene activity was decreased; the second screen yielded SCA1 modifiers when gene activity was increased. Both suppressors and enhancers of the neurodegenerative phenotype were obtained from each screen. The first aim of the proposed work is to identify the genes that modify the SCA1 neurodegenerative phenotype. These modifiers will be further characterized in sensitive viability and locomoter assays that allow the quantification of their modifier effects. The most powerful suppressors will be selected for further studies. To investigate whether different polyglutamine disease share common mechanisms of pathogenesis, the SCA1 modifiers will be tested in fly models of Huntington disease and polyglutamine toxicity. Finally, because the normal function of the SCA1 gene may be relevant to pathogenesis, the function of the Drosophila SCA1 gene will be investigated by generating lack-of-function mutations and transgenes for its over expression. In future studies, the most promising SCA1 suppressors characterized in flies will be investigated in the SCA1 mouse model, and in mouse models of polyglutamine disease. These genes may also be relevant to research aimed at treating other neurodegenerative proteinopathies such as Alzheimer disease and Parkinson disease. They will provide valuable targets for future pharmacological research aimed at developing drugs for therapy. -

Principal Investigator: BROADIE, KENDAL S
Grant Number: 5R01NS041740-05
Title: Synaptic Mechnisms in Drosophila Neurodegeneration Model

Abstract: The hypothesis driving this proposal is that presynaptic dysfunction is a common causative factor leading to cell death in multiple inherited neurodegenerative diseases. This hypothesis is based on the observations that 1) synaptic function mediates neuronal survival during development, 2) mutations which strongly impair presynaptic function result in massive, progressive neuronal degeneration, 3) a number of presynaptic proteins have been directly implicated in neurodegenerative diseases and 4) neuronal dysfunction/synapse loss is known to precede by a substantial period the manifestation of cell death in these diseases. To date, however, there is no established direct evidence of synaptic dysfunction mediating neuronal death during neurodegenerative disease states. The goal of this proposal is to assay synaptic maintenance in two genetic models of neurodegenerative diseases: Drosophila models of Parkinson's Disease (PD), a classic "protein storage" disease, and Niemann-Pick Type C (NP-C), a classic "lipid storage" disease. Drosophila was selected for its attractive properties as a new molecular genetic model of neurodegeneration, and its long history as the foremost genetic model for synaptic studies. PD and NP-C were selected as representative of a large number of related neurodegenerative disorders. The Drosophila PD model has been recently established through transgenic over-expression of human alpha-synuclein (a presynaptic protein) and shown to accurately recapitulate the diagnostic features of human PD. A Drosophila model of NP-C is being established through mutation (loss-of-function) of the endogenous NPC I gene, the known cause of human NP-C disease. Specifically, this proposal is to conduct age-progressive studies of synaptic mechanisms in Drosophila PD and NP-C models to correlate synaptic maintenance with the onset, progression and prevalence of neurodegeneration. The first aim is to improve Drosophila models by generating fluorescently tagged alpha-synuclein and NPCI proteins whose levels can be reversibly regulated through a temperature-dependent ubiquination strategy. Secondly, to confirm gross features of neurodegeneration in these models with behavioral assays and examination of nervous system/neuronal architecture. Third, and most importantly, to assay synaptic development, function and maintenance in these models. Assays will include electrophysiological measurements of neurotransmission, quantitative fluorescent optical imaging of protein and lipid dynamics in the presynaptic terminal and ultrastructural studies of presynaptic architecture. Together, these studies will allow a conclusive determination of whether synaptic maintenance is

Principal Investigator: BURKE, ROBERT E
Grant Number: 2P50NS038370-06
Title: Mechanisms of dopamine neuron degeneration

Abstract: Parkinson's disease (PD) is a prevalent and disabling neurological disease characterized by the progressive loss of motor control due to the degeneration of dopamine (DA) neurons of the substantia nigra. Among neurodegenerative diseases, PD has served as a model for the development of novel therapeutic approaches: administration of neurotransmitter precursors (levodopa), cell implantation, and more recently, deep brain stimulation. As important and effective as these advances have been, they only relieve symptoms; none stop the progression of the disease. In order to develop therapies which halt the progression of the disease, we need to achieve a better understanding of the pathogenesis of DA neuron degeneration. This submission represents a competing continuation application for a Morris K. Udall Parkinson's Disease Research Center of Excellence awarded to Columbia University in 1999. This renewal consists of four projects devoted to a single integrating theme: to understand the molecular and cellular mechanisms of dopamine neuron degeneration. While there are many worthy hypotheses of pathogenesis, the subprojects of this proposal will focus on four major current themes in the pathogenesis of PD, related to the roles of: (1) Abnormal intracellular protein degradation; (2) Inflammatory pathways; (3) Programmed cell death (PCD); and (4) Oxidative injury. In Project 1, Dr Serge Przedborski will evaluate the role of cyclooxygenase 2 (COX2) and cytosolic phospholipase A2 (cPLA2) (Theme 2) in mediating dopamine neuron damage in the MPTP model of PD and in human brain samples. In Project 2, Dr David Sulzer will examine in astrocyte and neuron primary cultures the role of chaperone mediated autophagy in the degradation of proteins implicated in PD (Theme 1) and the effect of these proteins on catecholamine sequestration (Theme 4). In Project 3, Dr Robert Burke will use genetic techniques in animal models to examine the roles of the mixed lineage kinases, Akt and JNK in mediating PCD in dopamine neurons (Theme 3), and he will evaluate the functional role of ER stress in initiating cell death (Theme 1). In Project 4, Dr Lloyd Greene will continue to evaluate the functional role of genes identified in the current funding period by SAGE analysis as upregulated following neurotoxin exposure. He will continue his studies of the role of ER stress-related genes (Theme 1) and genes implicated in PCD (Theme 3) in PC12 cells and primary sympathetic neurons, and in living animal models (the latter in collaboration with Drs Burke and Przedborski). He will also examine these transcripts and their protein products in PD brain. -

Principal Investigator: BURKE, ROBERT E
Grant Number: 5R01NS026836-15
Title: Apoptosis in substantia nigra

Abstract: The theme of our investigations has been that the molecular pathways of programmed cell death (PCD) may be relevant to the pathogenesis of Parkinson's disease and allied disorders. In recent years it has also become apparent that PCD regulates viability in cell-based therapeutic approaches, including tissue implants and neural stem cells. There has been tremendous growth in our knowledge of the molecular basis of PCD. However, most of this knowledge derives from relatively simple in vitro systems. While there are universal aspects of PCD mechanisms, it is nevertheless also clear that PCD is context dependent. It is therefore essential to translate this new knowledge to the in vivo context. A unique aspect of our approach is to examine PCD in postmitotic, phenotypically defined dopamine (DA) neurons in living brain. We will investigate two themes related to regulation and effector mechanisms of cell death in these neurons. While there is evidence that natural cell death (NCD) in DA neurons is regulated by striatum-derived neurotrophic support, the nature of these factors remains unknown. Theme 1 will examine the possibility that GDNF or neurturin (NTN) may serve as such factors. Our first Aim will examine the effect of increased striatal expression of GDNF, in a unique temporally-regulated bi-transgenic model, on the mature number of DA neurons. The second Aim will determine whether endogenous striatal GDNF regulates the magnitude of NCD in DA neurons, through the application of "knock down" approaches. Our third Aim will directly compare the potency of GDNF and NTN to suppress apoptotic death in a developmental axotomy model. Theme 2 will seek to identify important proteases mediating PCD in DA neurons in vivo. We have shown that activated caspase-3 is expressed in apoptotic DA neurons. In Aim IV, we will examine the functional significance of its expression, by studying the magnitude and protein cleavage characteristics of NCD, and the size of DA progenitor pools, in caspase-3 null animals. Many in vitro studies have shown that caspases-independent pathways exist. In Aim V, we will examine expression of the proteasome complex in PCD in DA neurons in vivo. The new knowledge gained by the studies outlined in this application will have direct implications for concepts of pathogenesis of Parkinson's disease and for approaches to optimizing cell-based treatments. -

Principal Investigator: CANAVIER, CARMEN C
Grant Number: 5R01NS037963-06
Title: Firing Pattern in Midbrain Dopamine Neurons

Abstract: This work seeks to understand the how the synaptic afferent inputs to midbrain dopamine neurons interact with their intrinsic properties to produce the range of firing patterns exhibited in vivo, and how these firing patterns exert their effects on the target neurons in the striatum. We will first produce a computer model of the dopamine neurons in vitro that replicates the effects of pharmacological manipulations on the regular spontaneous firing that characterizes dopamine neurons in the absence of afferent input, and provides insight into the mechanisms that convert this regular firing into burst firing or irregular firing. Then we will extend the model to the situation in vivo. The model will be used not only to elucidate the key currents, parameters, and mechanisms responsible for the generation and modulation of their electrical activity, but also to suggest therapeutic approaches for Parkinson's disease and other pathological conditions in which dopamine release plays a role. Currently such therapeutic strategies, including maximizing release from surviving or transplanted dopamine neurons, are limited by the inability to replace dopamine in the correct spatial and temporal pattern. Several lines of evidence indicate that not only the firing rate but also the firing pattern of these neurons is significant. Computational models supplemented by the techniques of nonlinear forecasting and nullcline analysis, will used to test our hypotheses about how various pharmacological agents exert their effects on the firing pattern of dopamine neurons, and how these changes in firing pattern might impact their targets in the striatum. We will identify model mechanisms and parameters responsible for characteristics of apamin and NMDA-induced burst firing such as variations in spike amplitude and interspike interval (ISI) as well as depolarization block, identify mechanisms responsible for irregular firing both in the model and in real neurons in vivo and in vitro, formulate a model of burst firing induced by synaptic excitation in vivo, and test our hypotheses regarding the functionality of irregular firing and the role of Dl receptor activation in focusing striatal activity. -

Principal Investigator: CHANG, JING-YU
Grant Number: 5R01NS045826-03
Title: Basal Ganglia Neurophysiology during DBS in Rats

Abstract: Parkinson's disease (PD) is a degenerative neurological disorder affecting millions of patients all around the world. Renewed use of the deep brain stimulation (DBS) method provides a new opportunity for treating PD. A key issue to improve the treatment is to fully understand the neural mechanisms underlying the therapeutic effects of DBS. In this proposed study, two unique techniques developed in our laboratory: the chronic multiple-channel single unit recording and rat model of DBS, will be employed to study the neural responses in multiple basal ganglia regions during behaviorally effective DBS in rat model of Parkinsonism. A first objective is to establish a rodent model of DBS in Parkinsonian conditions. The effects of DBS will be evaluated in dopamine lesioned rats performing treadmill locomotion and limb use asymmetry tests. Locomotor d deficits during treadmill walking and imbalance usage of forelimb in vertical exploratory behaviors will develop after unilateral dopamine lesion. High frequency stimulation (HFS) of the subthalamic nucleus (STN) and the substantia nigra pars reticulata (SNr) will then be applied to alleviate these motor abnormalities. The degree of dopamine depletion in the basal ganglia will be detected by immunohistochemical staining of dopamine marker and this result will be correlated with the severity of motor deficits and DBS effects. Second, the basal ganglia neural responses following a dopamine lesion and during behaviorally effective HFS will be examined. Single neural activity and local field potential in the striatum globus pallidus, STN and SNr will be recorded simultaneously in a 64 channel recording system in the rat performing these behavioral tests. Neural responses following dopamine lesion will help us to understand the pathophysiologic process of developing Parkinsonian syndromes while the neural responses during behaviorally effective HFS will shed light on how DBS can restore normal information processing in the basal ganglia neural circuits that are disrupted following dopamine lesion. Several important improvements on recording and stimulation techniques will be made in cooperation with Biographic Inc. to achieve optimal conditions for high frequency stimulation and artifact free recording. The goal of this study is to explore the basic neural mechanism underlying the therapeutic effects of DBS and the knowledge obtained form this study will help us to improve the clinical treatment of PD with DBS method. -

Principal Investigator: CHANG, JING-YU
Grant Number: 5R01NS043441-03
Title: Rat Model of Brain Stimulation in Parkinsonian Condition

Abstract: Deep brain stimulation (DBS) has been used in the clinic to treat Parkinson's disease (PD) during the past decade. The neuronal mechanisms underlying the therapeutic effects of DBS, however, are yet to be clarified. DBS methods have been developed based on the experiments performed exclusively on primate model. Many critical issues regarding the therapeutic effects of DBS need to be addressed using a rodent model. This proposal is aimed at three objectives: first is to establish a rodent model of DBS for Parkinsonian conditions. The rat will be subjected to unilateral 6-hydroxydopamine injection to destroy nigrostriatal dopamine system and thus develop a Parkinsonian motor deficit revealed by treadmill locomotion task. Treadmill will be turned on and off 20 seconds alternatively. Array of ten stimulation electrodes will be implanted in the subthalamic nucleus (STN) and substantia nigra pars reticulata (SNr). High frequency stimulation (HFS) will be applied during the treadmill walking phase. The improvement on locomotion by HFS will be measured and the effects will be compared between STN and SNr stimulations using different stimulation parameters. Second objective is to understand the dynamic neural activity responses in the basal ganglia system during the development of motor deficit by monitoring and comparing the activities from same neurons cross 10 day dopamine depletion period. Chronic multi- channel, single-unit recording technique will be used in this experiment. Sixty-four electrodes will be implanted in the striatum, globus pallidus, STN, and SNr. Extracellular spike activity will be recorded simultaneously in the behavioral rat. This study will test the hypothesis that direct and indirect pathways of basal ganglia will respond in different yet correlated manners during dopamine depletion. Third objective is to study the neuronal mechanisms mediating therapeutic effects of DBS in the behavioral model described above. In addition to the 64 electrodes implanted in the basal ganglia regions mentioned above, eight more stimulation electrodes will be added to target the STN and SNr. The neuronal responses in all four basal ganglia regions during behavioral effective HFS will be recorded and analyzed to reveal the effects of HFS on motor behavioral and associated changes in basal ganglia neuronal activity. This study is designed to address the fundamental mechanisms regarding the effects of DBS on treating PD and the information obtained from this experiment will have direct impact on improving the effects of DBS on PD and other movement disorders.-

Principal Investigator: CHEN, JIANG F
Grant Number: 5R01NS041083-05
Title: NOVEL BENEFIT OF A2A RECEPTOR INACTIVATION IN PD MODELS

Abstract: Parkinson's disease patients experience profound depletion of striatal dopamine (DA) due to degeneration of the nigrostriatal DA pathway. The predominant treatment for the past 30 years has been the DA precursor, L-dopa. While this strategy improves motor deficits, it has no effect on the underlying degenerative process, and indeed can have the additional unwanted side-effect of inducing dyskinesia. A possible alternative therapy, with neuroprotective ability appears to be use of antagonists of a specific class of adenosine receptors, A2A. These agents appear to have both motor-activating properties and preliminary data suggest they may also attenuate MPTP-induced DA neurotoxicity and prevent the locomotor stimulation that occurs with chronic DA receptor stimulation. The proposed studies will systematically investigate the novel motor and neuroprotective effects of A2A receptor antagonists. Methods center around pharmacological studies and use of genetic knockout (KO) approaches. There are three specific aims: 1) to test the hypothesis that A2A inactivation enhances motor function through D2R-dependent and independent mechanisms using A2AR-KO, D2R-KO and double KO mice; 2) to test the hypothesis that A2AR inactivation prevents the development of chronic L-dopa-induced rotational motor sensitization in unilateral 6-OHDA-lesioned mice; and 3) to characterize the role of V in MPTP-induced neurotoxicity by establishing the potency, "therapeutic window" and by "analyzing synergy between A2AR activation and inactivation;" in addition, the effect of A2AR agents on MPTP metabolism in vivo and in cell culture will also be examined to investigate the neurochemical mechanisms of protection by A2AR inactivation. -

Principal Investigator: Chu, Charleen T
Grant Number: 5R01NS040817-04
Title: THE PARKINSONIAN 6 HYDROXYDOPAMINE MODEL

Abstract: Parkinson's disease is the most common debilitating movement disorder of the aging human population. The neurons that degenerate in Parkinson's disease are subject to increased oxidative stress because superoxide and other reactive species are generated during dopamine metabolism. 6-hydroxydopamine (6-OHDA) is a redox cycling dopamine analog, which can be targeted to selectively damage the nigrostriatal system that degenerates in Parkinson's disease. Phosphotyrosine signaling pathways activated by neuroprotective factors, such as brain derived neurotrophic factor and glial cell line-derived neurotrophic factor, are important for dopaminergic neuron function and survival. This proposal is designed to investigate the hypothesis that oxidant-mediated alterations in phosphotyrosine signaling contribute to degeneration of dopaminergic neurons in Parkinson's disease. Nitrotyrosine, a marker of oxidative stress involving peroxynitrite formation, is increased in both the 6-OHDA rodent model and in human Parkinsonian brain tissues. Peroxynitrite is formed from the reaction of superoxide with nitric oxide, implicating these free radicals in the pathogenesis of Parkinson's disease. In this proposal, mechanisms by which 6-OHDA, superoxide, and nitric oxide affect phosphotyrosine signaling cascades will be investigated using immortalized dopaminergic neuron lines and mice with genetically altered levels of extracellular superoxide dismutase. This comprehensive set of studies will yield important insights concerning mechanisms by which oxidative stress affects neurotrophic signaling in dopaminergic neurons, potentially contributing to development of combined antioxidant-neurotrophic factor therapies for Parkinson's disease. -

Principal Investigator: COLLIER, TIMOTHY J.
Grant Number: 5R01NS042125-04
Title: Cell Grafts for Parkinson's Disease

Abstract: In vitro expansion of neural progenitor cells followed by induction of dopaminergic phenotype may provide a limitless source of cells for grafting into patients with Parkinson's disease (PD). However, the signals controlling the conversion of these cells into dopamine (DA) neurons must be identified. In an effort to accomplish this, single cells isolated from ventral mesencephalon were clonally expanded and exposed to hematopoeitic cytokines and neurotrophic molecules. Analysis of cell differentiation in response to this treatment yielded conversion of a high percentage (72 to 98 percent) of cells in some clones to a tyrosine hydroxylase (TH)-positive phenotype. Of the 24 clones generated, the best conversion to TH cells occurred with exposure to a combination of interleukin-1 (IL-1), interleukin- 11 (IL-11), leukemia inhibitory factor (LIF), and glial cell line-derived neurotrophic factor (GDNF). Positive clones expressed TH, the DA transporter, Nurr-1 and released DA in culture. Other cells in cytokine-exposed clones expressed GFAP (astrocyte marker) or MAP-2 (neuron marker) indicating that the original neurospheres were also capable of producing clones that differentiate into glial and nondopaminergic neurons. Initial neural grafting studies m the rat model of PD using a clone with the highest conversion rate to TH indicated that converted progenitor cell grafts produced complete amelioration of amphetamine-induced rotational behavior and continued to express the TH phenotype. However, the survival rate of these grafted progenitor cells was reduced (26 percent) compared to embryonic ventral mesencephalon (VM). The experiments proposed here will develop protocols for optimal survival of Wafted cytokine-converted mesencephalic progenitor cells. Once survival of grafted mesencephalic progenitor cells is optimized, direct comparisons will be made to fresh embryonic VM grafts on measures of behavior, in vivo dialysis, post-mortem DA biochemistry, DA receptors, cell survival and neurite extension. Lastly, this proposal will test the efficacy of the DA conversion cocktail on clonal progenitors derived from embryonic mesencephalon of nonhuman primate brain. If successful, cytokine-converted mesencephalic progenitor cells could potentially replace embryonic tissue as the primary source of cells for grafting in PD. -

Principal Investigator: Corcos, Daniel M
Grant Number: 5R01NS028127-10
Title: MOTOR DEFICITS - EXPERIMENTAL AND CLINICAL CORRELATES

Abstract: Parkinson's disease is a progressive neurological disease that dramatically alters the ability of individuals to move. The long-term objective of the proposed research program continues to be to understand how Parkinson's disease changes the way muscles are adapted to perform movements. Aim 1 will test the hypothesis that Parkinson's disease causes an inability to: 1) turn off muscles that are activated, 2) prolong muscle activation and delay antagonist muscle activation for longer movements and, 3) use appropriate patterns of muscle activation to adapt to unexpected changes in load. Aim 2 will determine the extent to which the imbalance between flexor and extensor muscles impairs motor function. The hypothesis is that the electromyographic (EMG) abnormalities address in Aim 1 will be greater in extensor muscles than in flexor muscles. Aim 3 will determine whether disease severity influences the patterns of muscle activation observed in patients with Parkinson's disease. The hypothesis is that disease severity does influence patterns of muscle activation. A second hypothesis is that certain EMG deficits will appear successively in all subjects, while others will appear in a different order in different subjects related to the particular manifestation of the disease. For example, the Principal Investigator expects to see a loss of agonist EMG burst during modulation followed by a decrease in latency of the first antagonist EMG burst. In contrast, the difficulty in turning off muscle activation may not emerge in a fixed relation to the loss of agonist during modulation. Studying two groups of patients who have different degrees of disease severity will test both these hypotheses. The experiments have been carefully chosen to build upon prior studies of muscle activation patterns in neurologically normal individuals, and in patients with Parkinson's disease. Aims 1 and 2 will provide information that can be used to develop models of motor control that apply to a wide variety of movements in Parkinson's disease. Aim 3 will allow the investigators to determine the extent to which EMG and performance deficits manifest themselves at all stages of the disease. Understanding how much muscle activation patterns change to perform movements is important in evaluating pharmacological, neurological, as well as physical therapy interventions that are designed to facilitate movement in Parkinson's disease. -

Principal Investigator: DAUER, WILLIAM T
Grant Number: 1K02NS045798-01A1
Title: The mechanism of MPTP resistance in synuclein null mice.

Abstract: My long-held career goal is to investigate questions of importance to both patient care and fundamental biology. During medical training, I developed a strong interest the basic pathogenic mechanisms of Parkinson's disease (PD), an illness characterized by degeneration of substantia nigra dopamine (DA) neurons and cytoplasmic aggregates of alpha-synuclein (SYN). I came to appreciate the power of genetically modified animals as tools to explore basic aspects of disease pathogenesis, and developed expertise in the generation of such animals. However, I now need to acquire skills necessary to assess the consequences of PD-related mutations on cellular and behavioral aspects of dopaminergic function in these animals. To accomplish this goal, I have developed collaborations with experts in PD research, and will pursue the proposed work within the integrated PD research group at Columbia University. Rarely, PD may be caused by missense mutations in SYN. However, normal SYN function and the mechanism by which pathogenic mutations disrupt SYN biology and lead to PD are poorly understood. MPTP-induced degeneration of DA neurons is a commonly studied model of PD. We find that SYN null mice display striking resistance to MPTP-induced degeneration of DA neurons, and this resistance appears to result from an inability of the toxin to access and inhibit its target, mitochondrial complex I. The goal of this research plan is to exploit this robust phenotype of SYN null mice to gain insight into the normal function of SYN, and explore how this function is altered by PD-causing mutations. In Aim 1 we will measure whether known concomitants of complex I inhibition (increased lactate and reactive oxygen species; decreased ATP) are also impaired in SYN null mice, and characterize processes that control access of the toxin to complex I (vesicular and monoamine transporter function). In Aim 2 we will further explore whether altered synaptic function underlies the MTPT resistance of SYN null mice by testing whether they are selectively resistant to toxins that traffic through the synapse. In Aim 3, by restoring wild type or mutant SYN to specific neuronal populations of SYN null mice, we will test whether the MPTP resistance is a cell autonomous phenomenon and whether pathogenic SYN mutations modify an aspect of its function involved in effecting MPTP-induced neurodegeneration. This proposal exemplifies the type of clinically related fundamental neurobiological research I plan to pursue during my career.-

Principal Investigator: DAWSON, TED M
Grant Number: 1R21NS047565-01
Title: Models of Familial Parkinson's Disease: DJ-1 Knockouts

Abstract: Mutations in the DJ-1 gene are a rare genetic cause of autosomal recessive Parkinson's disease (PD). The DJ-1 protein is either absent or appears to be functionally inactive in the families in which mutation have been identified. Thus, mutations in the DJ-1 gene probably cause PD through a loss of function. It is difficult at this juncture to fully appreciate how mutations in the DJ-1 gene cause PD, as its function is largely unknown. DJ-1 was identified as a hydroperoxide-responsive protein that becomes more acidic following oxidative stress suggesting that it may function as an antioxidant protein. Furthermore, DJ-1 is sumoylated through binding to the SUMO-1 ligase, PIAS, suggesting that it might be involved in the regulation of transcription. Other putative functions of DJ-1 have been raised, but how a loss of function of DJ-1 leads to loss of DA neurons and PD awaits further study. We propose to generate and characterize DJ-1 knockout mice to formally test the hypothesis that the absence of DJ-1 function is the cause of PD due to DJ-1 mutations. Accordingly experiments are proposed to further characterize the role of DJ-1 in the pathogenesis of PD. In Specific Aim #1 we will develop and characterize DJ-1 knockout mice. In Specific Aim #2 we will evaluate the sensitivity of DJ-1 knockouts to environmental toxins including MPTP-induced dopaminergic cell death. In Specific Aim #3 we will determine whether DJ-1 interacts with parkin by evaluating the effect of crossing DJ-1 knockout mice with parkin knockout mice. Development and characterization of DJ-1 knockouts, understanding the relationship of DJ-1 and parkin in the pathogenesis of PD may provide insight into the molecular mechanisms by which these gene products induce neuronal damage and may provide novel therapeutics and targets to prevent the toxic effects of these familial associated genes in the degenerative process of PD. -

Principal Investigator: DAWSON, TED M
Grant Number: 1R01NS048206-01
Title: The Role of Parkin in Parkinson's Disease

Abstract: Mutations in the parkin gene are the main genetic cause of autosomal recessive Parkinson's disease (PD) and mutations in parkin also play a major role in familial Parkinson's disease. Preliminary studies indicate a potential pivotal role for parkin in the ubiquitin proteasomal pathway (UPP) by functioning as an ubiquitin E3 ligase. Most disease causing mutations of parkin are thought to be loss of function mutations that ultimately lead to the absence of ubiquitination and the subsequent failure of UPP-mediated degradation of parkin substrates. Thus, the abnormal accumulation of parkin substrates is thought to play a role in the demise of substantia nigra dopaminergic neurons in patients with parkin mutations. A number of putative parkin substrates have been identified, but their importance in the pathogenesis of PD due to parkin mutations is not known. We propose to generate and characterize parkin knockout mice to formally test the hypothesis that the absence of parkin function is the cause of PD due to parkin mutations. Furthermore, biochemical and proteomic characterization of the parkin knockout mice may shed light on the substrates that are important in the pathogenesis of PD due to parkin mutations. Accordingly experiments are proposed to further characterize the role of parkin and it's substrates in the pathogenesis of Parkinson's disease. In Specific Aim #1 we will develop and characterize parkin knockout mice. In Specific Aim #2 we will evaluate the sensitivity of parkin knockouts to environmental toxins including MPTP-induced dopaminergic cell death. In Specific Aim #3 we will evaluate the interaction of parkin with the alpha-synuclein interacting protein, synphilin-1 and determine whether parkin mediates K48 or K63 ubiquitin linkages. In Specific Aim #4 we will determine whether parkin interacts with alpha-synuclein and evaluate the effect of crossing parkin knockout mice with A53T mutant alpha-synuclein transgenic mice. In Specific Aim #5 we will identify and characterize parkin interacting proteins in parkin knockout mice. Development and characterization of parkin knockout mice, understanding the relationship of parkin, alphasynuclein and synphilin-1 in the pathogenesis of PD may provide insight into the molecular mechanisms by which these gene products induce neuronal damage and may provide novel therapeutics and targets to prevent the toxic effects of this familial associated genes in the degenerative process of Parkinson's disease. -

Principal Investigator: DAWSON, TED M
Grant Number: 2P50NS038377-06A1
Title: Parkinson's Disease Research Center of Excellence

Abstract: The overall goals of this proposal are to understand the role of alpha-synuclein, parkin, DJ-1 and synphilin-1 in the pathogenesis and pathology of Parkinson's disease (PD) and to define the molecular mechanisms of neuronal injury in animal models of PD. The program represents a multi-disciplinary, mechanistic approach involving interactive, productive investigators with complementary areas of expertise who have long been committed to the studies of neurodegenerative diseases. Their aim will be to integrate the activities of various disciplines such that the interrelationships will result in a greater scientific contributions and achievements if each project were pursued individually. The program has one major theme: To understand the role of familial associated genes alpha-synuclein, parkin and DJ-1 in the pathogenesis of Parkinson's disease and related disorders. The role of alpha-synuclein, parkin, DJ-1 and synphilin- 1 in PD pathogenesis will be investigated using molecular, transgenic, neuropathologic, cell biologic and neurobehavioral approaches to examine the mechanism of neuronal dysfunction and injury clue to alterations in these gene products. The mechanism of neuronal loss in Parkin knockout mice and alpha-synuclein A53T transgenic mice will be characterized. We will determine whether parkin interacts with alpha-synuclein and further explore the relation between and parkin, alpha-synuclein and synphilin-1. We will explore alpha-synuclein processing and modifications and the relationship of synphilin-1 to alpha-synuclein. Furthermore, we will investigate the potential function of DJ-1 and it role in PD Pathogenesis. We believe that our multi-disciplinary approach has the capacity to produce unique information concerning the mechanisms of neurodegeneration in genetic animal models of Parkinson's disease and the related synucleinopathies and to lead to better understanding of the function and the role of alpha-synuclein, parkin, DJ-1 and synphilin-1 in normal and pathophysiologic processes related to PD. The program consists of four projects: 1) Mouse Models of Parkin Biology and Pathobiology 2) PD Cell Models: Alpha-synuclein and Interacting Proteins; 3) Mechanisms of Neurodegeneration in Human Alpha-synuclein Transgenic Mice; 4) The Role of DJ-1 in Parkinson's Disease and four cores A) Administration and Training; B) Transgenic and Neurobehavior; C) Neuropathology and D) Clinical.-

Principal Investigator: DEBBURMAN, SHUBHIK
Grant Number: 1R15NS048508-01
Title: Yeast Model for Two Neurodegeneration-Linked Proteins

Abstract: Budding Yeast (S. cerevisiae) has emerged as a powerful model system for understanding molecular aspects of many human diseases. Protein misfolding linked to certain neurodegenerative diseases (NDDs) like Huntington Disease, Lou Gehrig's disease, and prion diseases have been successfully recapitulated in S. cerevisiae and led to identification of therapeutically relevant regulators of misfolding. No S. cerevisiae models for Parkinson's Disease (PD) or dentatorubral pallidoluysian atrophy (DRPLA) have been reported. PD is one of the most common NDDs, while DRPLA is a rare inherited NDD of the triplet repeat disease family. In both diseases, misfolding of a specific protein (alpha-synuclein for PD and atrophin for DRPLA) is thought to cause selective neuronal death. Unlike the well-characterized huntingtin protein in Huntington Disease (which shares many similarities to DRPLA), less is known about the misfolding of mutant atrophin in DRPLA. A S. cerevisiae expression system for studying alpha-synuclein has recently been developed in our lab. Preliminary evidence supports that both wildtype and disease-associated mutants are aggregating within yeast cells and upon purification. A similar effort to establish atrophin-1 expression in yeast is underway. To extend initial observations with alpha-synuclein in yeast and fully develop a yeast model for atrophin, three goals are proposed. 1) Misfolding properties between wildtype and mutant versions of both proteins will be investigated in vivo (immunofluorescence and GFP-based localization and assessment of protein half-life) and in vitro (by measuring protease sensitivity and differential solubility). 2) Influences of chaperones and ubiquitin-proteasomal pathway proteins on folding and degradation of these proteins will be assessed in strains compromised for chaperone/proteasomal function, or those that overexpress chaperones, and by co-immunoprecipitation assessment. 3) A fission yeast (S. pombe) expression model for alpha-synuclein and atrophin properties (as in Aim 1) will be developed and compared with the S. cerevisiae model; NDD models have not been reported in S. pombe. These studies may further clarify the molecular bases for misfolding and degradation of PD- and DRPLA-linked proteins and extend the usefulness of yeast models. Importantly, the scientific training of many undergraduates will be supported, strengthening their cell biology and molecular genetics skills and appreciation for model organisms. -

Principal Investigator: DEBBURMAN, SHUBHIK
Grant Number: 3R15NS048508-01S1
Title: Yeast Model for Two Neurodegeneration-Linked Proteins

Abstract: Unavailable

Principal Investigator: DENG, HAN-XIANG
Grant Number: 5R01NS040308-04
Title: TRANSGENIC STUDIES OF AMYOTROPHIC LATERAL SCLEROSIS

Abstract: The goal of this project is to investigate the pathogenic mechanisms underlying the neurodegeneration of amyotrophic lateral sclerosis (ALS) using transgenic mouse models. Our application has two immediate Aims: The first one is to replace the two free cysteine residues in mutant human SOD 1 protein to test the role of free -SH groups in the formation of intracellular aggregates noted in ALS. The second aim is to define the smallest fragment of SOD 1 that would still cause ALS in transgenic mice. AIM 1: About 20 percent of the familial ALS cases are caused by mutations in Cu/Zn superoxide dismutase gene (SOD 1). Transgenic mice that over express mutant SOD 1 develop an ALS-like phenotype and motor neuron degeneration. A common feature in the pathology of both human SOD1-linked ALS and the ALS (SOD1) mouse models is the presence of the SOD 1-inimunoreactive inclusions or aggregates in neurons of the brain and spinal cord. These inclusions/aggregates are thought to be important elements in motor neuron death in ALS. To test the hypothesis that these inclusions/aggregates may be formed by disulfide bonds through interaction of free -SH groups of one or both free cysteines in SOD 1, we propose to develop new transgenic mouse model that over expresses a mutated SOD 1 (C6A/C1 1 1S/G93A). In this transgenic mouse model, two free cysteines in human SOD1 are replaced by an alanine and a serine. Absence of inclusions/aggregates will indicate a role for free cysteines; Absence of disease as well as inclusions/aggregates may indicate a causative role of the free cysteines (-SH groups). AIM 2: We recently made a new transgenic mouse model that over expresses a truncation mutation in SOD 1 (L126Z). These mice developed a typical ALS-like phenotype and pathology. These results provide the experimental evidence that only a part of the SOD1 polypeptide, rather than entire SOD1 protein, is sufficient to produce the neuronal toxicity and cause motor neuron degeneration. We plan to define the minimum fragment of SOD 1 essential for toxicity so that further studies into the pathogenesis of ALS may be facilitated. To achieve this goal we propose to develop additional transgenic mouse lines that over express successively smaller fragments of SOD 1 polypeptide to determine the smallest segment of SOD 1 that causes ALS. -

Principal Investigator: DESHMUKH, MOHANISH
Grant Number: 5R01NS042197-04
Title: Mechanism of Neuronal Competence To-Die-By Apoptosis

Abstract: Programmed cell death (PCD), which results in apoptosis, occurs widely during neuronal development and is also observed in pathological situations of stroke, spinal cord injury, and neurodegenerative disease. The mechanism of neuronal PCD has been extensively studied in sympathetic neurons that undergo apoptosis after nerve growth factor (NGF) removal in culture. A critical factor regulating apoptosis in many cells is the cytochrome c-dependent activation of caspases. Although necessary in sympathetic neurons, cytochrome c release is not sufficient to induce apoptosis after NGF deprivation. We have recently demonstrated that a novel, uncharacterized event, called the "development of competence," is needed, along with cytosolic cytochrome c to induce caspase activation and apoptosis in these neurons. We shall examine whether the development of competence event is also important in other models of neuronal apoptosis and test the specific hypothesis that the requirement of development of competence to induce apoptosis is a phenomenon unique to postmitotic cells. We shall also examine the signaling pathway activated after NGF deprivation that leads to the development of competence. Since our preliminary results suggest that the c-jun-N-terminal kinase (JNK) signaling pathway is important in regulating competence in sympathetic neurons, we shall focus specifically on components of this signaling pathway. Lastly, we shall examine the molecular mechanism of development of competence. Our recent data suggest that competence may be controlled by an inhibitor of apoptosis protein (IAP) like activity. We shall examine this hypothesis and test the specific importance of Smac, a recently identified inhibitor of IAPs, in regulating the development of competence in neurons. These studies will provide an understanding of the biological importance and mechanism of development of competence in promoting neuronal apoptosis. Knowledge of this pathway may also identify targets for the development of strategies to suppress apoptosis and ameliorate the consequences of neuronal injury and neurodegenerative disease. -

Principal Investigator: DIXON, C EDWARD
Grant Number: 3R01NS033150-09S1
Title: CHRONIC CHANGES IN NEUROTRANSMISSION FOLLOWING TBI

Abstract: Unavailable

Principal Investigator: DRISCOLL, MONICA A
Grant Number: 5R01NS041632-04
Title: C. elegans transporters in neuronal function

Abstract: Glutamate is an excitatory neurotransmitter essential for function of the nervous system. Certain injurious conditions cause excess glutamate to accumulate at the synapse, resulting in hyperstimulation of the post-synaptic neuron that can cause cell death. Glutamate transporters play an essential role in neuronal health and function by removing excess glutamate from the synaptic cleft. Considerable evidence implicates defective glutamate transport in ALS. It is particularly striking that approximately 65% of sporadic ALS patients have been reported to express aberrant glutamate transporter transcripts in affected neurons, a phenotype correlated with inhibition of glutamate transport. We propose to conduct a thorough analysis of glutamate transporter mutations in the facile C. elegans model system. The genome of this animal (sequenced to completion) encodes six glutamate transporter genes. We will determine when, and in which cells, the transporters are expressed, we will characterize their biochemical properties, and we will determine the loss of function phenotypes of each. We will also analyze the effects of expressing aberrant transporter transcripts, analogous to some produced in ALS patients, in transgenic animals. Finally, we will exploit the full power of C. elegans genetic analysis to conduct screens for new mutations that reverse or modify transporter defects. Genes identified in these screens should advance understanding of both normal and aberrant glutamate signaling. Since the C. elegans model system offers several unique advantages and most biological processes are conserved, we expect that results of the proposed study should provide new insight into basic mechanisms of Glu regulation at the synapse and may suggest novel strategies for preventing neurodegeneration in ALS. -

Principal Investigator: DUGAN, LAURA L
Grant Number: 5R01NS041796-04
Title: UCP5-- Balancing Metabolism and Oxidation in Aging Brain

Abstract: Age is the single greatest risk factor for most neurodegenerative disorders, even those that are genetically based. This delayed onset is believed to reflect an interaction between the risk factors for a neurodegenerative disease, and the aging process itself. Oxidative damage to mitochondrial DNA accumulates in brain of older individuals in many species, including man. This observation has led to the speculation that oxidative injury to mitochondria causes loss of mitochondrial metabolic reserve during aging, and that this contributes to the age-dependent onset of neurodegenerative processes. One class of proteins uniquely situated to contribute to, or modify, these age-dependent changes in mitochondrial function are the mitochondrial uncoupling proteins (UCPs). Mitochondrial uncoupling proteins are specifically designed to impair the efficiency of energy production by mitochondria to produce heat. Outside the nervous system, UCPs regulate body weight, temperature, and the response to starvation. Recently, however, we and others have shown that these proteins also regulate mitochondrial free radical production. Three UCPs (UCP2, 4, and 5) are expressed in brain, where their function(s) is essentially unknown. Our laboratory has been studying UCP5, and has determined that it is a neuronal protein with high expression in the forebrain of both mouse and man. We also found that over-expression of UCP5 in neurons decreased mitochondrial free radical production, a potentially beneficial effect, but decreased the efficiency of mitochondrial function and enhanced the vulnerability of neurons to injury and subsequent degeneration. We hypothesize that UCP5 in brain may be a two-edged sword which trades lower mitochondrial free radical production for greater mitochondrial metabolic inefficiency. We propose to determine whether expression and/or activity of UCP5 is altered in brain during aging. We will also determine whether this results in 1) constitutively higher levels of free radical production by mitochondria in older brain, and 2) increased vulnerability of brain to metabolic stress when UCP5 expression is induced. We will first identify factors, such as hormones or caloric restriction, which regulate expression and activity of UCP5. We will then use biochemical and fluorescence imaging techniques to evaluate mitochondrial function and free radical formation. Initial experiments will be performed in cultured neurons with modified levels of UCP5 or after treatment with agents to modify UCP5 levels or activity. We will then look at how altering UCP5 expression/activity impacts mitochondrial function and free radical production in brain of old mice. For many of these experiments, we will use Thy1-YFP mice,

Principal Investigator: DUNAH, ANTHONE W
Grant Number: 1K01NS049006-01
Title: REGULATION OF NMDA RECEPTOR TRAFFICKING BY DOPAMINE

Abstract: This grant is a request for a NINDS Career Development Award for Minority Scholars in Neuroscience (K01) to investigate the Regulation of NMDA Receptor Trafficking by Dopamine. Interactions between the dopaminergic and glutamatergic systems in the striatum have implications for the pathogenesis and treatment of Parkinson's disease. My previous work has revealed significant modifications in the properties of striatal NMDA glutamate receptors in animal models of Parkinson's disease. Intriguingly, the alterations in striatal NMDA receptors occur at the level of assembly, phosphorylation and synaptic localization of the subunit proteins, and involved redistribution of receptors between sub-cellular compartments. Furthermore, we recently reported evidence for a rapid dopamine D1 receptor dependent mechanism for the trafficking of striatal NMDA receptors from intracellular compartments to the post-synaptic membrane. The molecular mechanisms for the dopamine D1 receptor mediated sub-cellular trafficking of NMDA receptors in the striatum remain largely unknown. Therefore, I will apply my molecular neuroscience and neuropharmacology backgrounds to experimentally explore and unravel the dopamine receptor dependent molecular mechanisms and signaling pathways underlying the trafficking of striatal NMDA glutamate receptors to brain synapses in primary cell culture system. As a research fellow, I have gained knowledge and received proper training in molecular mechanisms of dopamine and glutamate mediated signal transduction pathways in both in vivo and in vitro systems. The proposed career development program will further my understanding of how the dopamine and glutamate systems in the striatum interact and lead to the pathogenesis of Parkinson's disease. This career development program along with my assembled team of scientists will continue to contribute to my professional and intellectual growth, and eventually establish myself as an independent investigator. The findings from this research proposal may ultimately lead to the development of new therapeutic options for human Parkinson's disease.-

Principal Investigator: DURING, MATTHEW J
Grant Number: 1R01NS044576-01
Title: Somatic Cell Gene Transfer/Neurological & Clin Applics

Abstract: Gene transfer in the mammalian nervous system has been the primary research focus of our laboratory for the past decade. We are excited that this RFA has come at a time when the field is flourishing, yet clinical translation remains daunting, and much work needs to be done for ultimate success in the clinic. In this grant application we propose to focus on some of the more pressing needs using rat models of Parkinson Disease. Our first aim is to further develop more efficient and readily packaged and purified AAV vectors for clinical translation. Here, we will characterize and compare pseudotyped and chimeric AAV vectors and in addition develop novel reagents, including helper plasmids and protocols which can be used by the entire gene therapy community to more efficiently generate these vectors. Our preliminary data suggests that these new chimeric and pseudotyped vectors represent a significant advance above our current generation rAAV-2 vectors. Secondly, we will develop optimal expression cassettes with a focus on promoter; post regulatory sequences as well as elements like the human beta-interferon scaffold attachment region (SAR) to boost expression. Thirdly, we will further develop a regulatable system. We present in our preliminary data our latest generation bi-directional tet cassette with tandem minimal insulator sequences flanking the vector genome. Here we propose to use this vector as the starting point to develop a novel cassette with the use of KRAB-AB domain from kid-1 as a suppressor. Our fourth aim is the use of rAAV to over express PAEL receptor in the adult rat substantial nigra with characterization of the phenotype as a potential genetic model of Parkinson Disease. Finally, we propose the use of a picospritzer and in vivo single unit recording to develop methods for focal and electrophysiological mapped neuronal gene delivery. We will target the substantia nigra pars compacta, using AAV expressing wildtype parkin, as a potential therapy for parkin mutation associated, autosomal recessive Parkinson Disease (AR-PD) as modeled by the PAEL receptor over expressing rats as developed in specific aim 4.-

Principal Investigator: EAKIN, CATHERINE M
Grant Number: 5F31NS046937-02
Title: Mechanisms of divalent cation associated amyloidosis

Abstract: The conversion of normally soluble proteins into amyloid fibers has pathological and functional consequences in a number of human diseases. A general cause for amyloid formation is not known. However, in many types of fiber formation, interaction of the protein precursors with divalent metals promotes aggregation. Divalent metals, particularly Cu2+, have been implicated as a central component in the formation of amyloid fibers in an increasing number of diseases. These include amyloid-beta in Alzheimer's, prion protein in Creutzfeldt-Jakob Disease, immunoglobulin light chain in Light Chain Amyloidosis, alpha-synuclein in Parkinson's, and beta-2-microglobulin (beta2m) in Dialysis Related Amyloidosis (DRA). Interaction with divalent metals may act to induce novel structure, preferentially bind amyloidogenic intermediates, or catalyze the sampling of a refolding pathway which contains amyloidogenic intermediates. The experiments proposed here will investigate the molecular basis for divalent metal associated amyloid formation in DRA. The first aim of this work is to determine the kinetics and pathway of Cu2+ associated fibrillogenesis of beta2m. The second aim is to determine the structure of a well-defined amyloidogenic precursor formed in the presence of Cu2+. The third aim is to determine the unfolded state structure of beta2m formed in the presence of divalent metal. These experiments will aid in understanding amyloid formation in DRA, but also contribute to establishing a general model for divalent metal associated amyloid formation.-

Principal Investigator: ELSINGER, CATHERINE L
Grant Number: 1R43NS049705-01
Title: fMRI Evaluation of Parkinson's Disease

Abstract: Parkinson's disease (PD) is a progressive and incurable neurological disease affecting an estimated 4 million people worldwide. Health care costs in the U.S. alone have been estimated in excess of $6B. While many FDA-approved therapeutic interventions (pharmaceutical, surgical and physiological) have become available for the management of the motor and cognitive complications associated with PD, the majority of interventions become less effective over time as the disease progresses. The challenge is to develop more effective and longer lasting treatments that alter the disease course in addition to managing symptoms. Identifying incremental therapeutic efficacy over existing treatments may be hindered by existing clinical outcome measures that suffer from relatively low reliability and sensitivity. The next wave of clinical trials, therefore, will likely require reliable and sensitive biological markers that correlate with clinical outcomes. In Phase I of this project, we propose to test the efficacy of functional magnetic resonance imaging (fMRI), as a biomarker for quantifying a therapeutic response in PD. Phase II will entail the development of a standardized neuroimaging platform based on proprietary technology to be implemented across wide range of MRI scanner platforms. This commercial platform will target academic medical centers, hospitals, and clinics, as well as the pharmaceutical industry, in order to facilitate the evaluation of therapeutic response in PD. -

Principal Investigator: ESKANDAR, EMAD N
Grant Number: 2K08NS041851-04
Title: Neostriatal Visual Processing & Initiation of Movement

Abstract: The basal ganglia are a group of subcortical nuclei that are important for motivation and motor control. Disorders of the basal ganglia lead to a variety of disabling movement disorders, the most common of which is Parkinson's disease. The input nuclei of the basal ganglia in primates include the caudate and putamen. The output nuclei include the Gpi and the substantia nigra pars reticulata. Other important nuclei include the substantia nigra pars compacta and the subthalamic nucleus. The input and output nuclei of the basal ganglia are joined by two distinct sets of connections, known as the "direct" and "indirect" pathways. The current model of basal ganglia function holds that the two pathways are in functional opposition and that activation of the direct pathway facilitates movement while activation of the indirect pathway inhibits movement. This explanation works well in empirically explaining what areas of the two pathways are overactive in movement disorders. For example, the Gpi and STN are overactive in PD and hence are effective targets for treatment. However, the nature of the interaction between the two pathways is poorly understood. Most recent models of the basal ganglia emphasize their role in suppressing unwanted movements although this has never been directly tested. Therefore, the primary goal of this research is to understand the role of the basal ganglia in suppressing unwanted movements by recording the activity of basal ganglia neurons in awake behaving primates trained in a movement suppression task. The second goal is to compare the data obtained in primate studies with information obtained by recording from the subthalamic nucleus and globus pallidus of patients undergoing surgery for the treatment of Parkinson disease. In this fashion we hope to understand the derangements of basal ganglia function which occur in PD and to devise better treatment strategies. This work will be conducted in the Department of Neurobiology at Harvard Medical School and in the Department of Neurosurgery at Massachusetts General Hospital.-

Principal Investigator: FEANY, MEL B
Grant Number: 5R01NS041536-04
Title: Drosophila Model of Parkinson's Disease

Abstract: Parkinson's disease is a common neurodegenerative syndrome characterized by loss of dopaminergic neurons in the substantia nigra, formation of filamentous intraneuronal inclusions (Lewy bodies), and an extra pyramidal movement disorder. Although several genes involved in familial Parkinson's disease have recently been identified, we still know very little about the molecular and biochemical events mediating neuronal dysfunction and death of dopaminergic neurons. To enable a comprehensive genetic analysis of Parkinson's disease, we have developed a Drosophila melanogaster model of the disorder. Expression of human a-synuclein in transgenic flies replicates the three cardinal manifestations of the human disease: adult-onset loss of dopaminergic neurons, filamentous intraneuronal inclusions containing a-synuclein, and progressive locomotor dysfunction. We now propose to exploit the genetic potential of the system by generating second site suppressors and enhancers of a-synuclein mediated neurodegeneration. A robust and titratable retinal phenotype suitable for genetic modification has been defined. Existing collections of well-defined mutant chromosomes will he assayed for their ability to modify the retinal phenotype. De novo mutations will also be generated and tested. Mutations that modify the retinal phenotype will be tested for their ability to alter dopaminergic neurodegeneration and inclusion formation. Modifiers of neurodegeneration and inclusion formation will be characterized molecularly. Mammalian homologues of these Drosophila modifiers will he human disease gene candidates and likely components of mammalian neurodegenerative pathways. We will also test the role of the ubiquitin/proteosome system, chaperones, and apoptosis in dopaminergic neurodegeneration using genetic methods. The role of the ubiquitin system and heat shock proteins will also be tested by looking for the presence of these proteins in Drosophila asynuclein aggregates. Ubiquitin co-localization studies will further address the relevance of the Drosophila system to human disease, because ubiquitination is a pervasive feature of human Lewy bodies. We can abolish inclusion formation in a-synuclein transgenic flies, and will determine if inclusions are required for neurotoxicity. -

Principal Investigator: FENG, JIAN
Grant Number: 5R01NS041722-04
Title: Parkin--In vivo Function and Role in Parkinson's Disease

Abstract: Parkinson's Disease (PD) is one of the most frequent neurodegenerative disorders. It is an extrapyramidal movement disorder characterized by the progressive loss of dopamine (DA) neurons in substantia nigra (SN). Recent progress in linkage studies on patients with familial PD has led to the identification of several genes implicated in this disease. One of these genes, parkin, is linked to Autosomal Recessive-Juvenile Parkinson's Disease (AR-JP). Deletions, truncations, and point mutations of parkin in AR-JP patients are correlated with their PD symptoms. However, it is not clear whether the loss of function for this gene directly causes PD, and if so, how does it occur? We propose to answer these questions by generating the parkin knockout mice. They will be used to study whether the deletion of parkin directly leads to selective loss of nigral DA neurons, and PD-like symptoms in mice. Since parkin is widely expressed in many tissues, and yet the progressive cell death is restricted to DA neurons in SN, we hypothesize that parkin may interact with specific proteins in these neurons to sustain their survival. To test this hypothesis, we propose to identify proteins that interact with parkin by using the yeast two-hybrid system. Once these proteins are identified, we will investigate their roles, in association with parkin, in the survival of nigral DA neurons. The specific goal of this project is to understand the in vivo function of parkin and its role in the etiology of AR-JP. As AR-JP and the sporadic form of PD share many similar clinical symptoms and pathological hallmarks (e.g. death of nigral DA neurons), knowledge gained from the study of parkin may shed some light on the potentially common mechanism for PD. It is our long-term objective to use this mouse genetic model to elucidate the molecular and cellular processes that lead to the progressive and selective death of nigral dopaminergic neurons and locomotor dysfunction in PD. This animal model would also be a valuable tool for the development of more effective therapeutic strategies for PD patients. -

Principal Investigator: FRIEDLANDER, ROBERT
Grant Number: 5R01NS041635-03
Title: Mechanisms and modulation of disease progression in ALS

Abstract: The functional role of the caspase cell death family in neurodegeneration, in particular ALS, has been clearly demonstrated. We have shown that caspases-1 and -3 are regulated at the transcription level in the mutant SOD1G93A transgenic ALS mouse model. Caspases-1 and -3 are specifically activated in ventral horn neurons in this mouse model. Adding relevancy to this finding, caspase-1 and -3 activation have been demonstrated in spinal cord of humans with ALS. Caspase inhibition, either by the caspase-1 dominant negative transgene, or by administration of the broad caspase inhibitor zVAD-fmk, slows disease progression and delays mortality in mutant SOD 1G93A mice. The broad goal of this study is to expand our understanding of the molecular and cellular pathways mediating neuronal cell death. This knowledge should contribute to the rational development of improved therapeutics for ALS. With this goal in mind we wish to evaluate the cell autonomous and non-cell autonomous signals modulating disease progression in ALS. The aims of this study include: 1) Evaluate the non-cell autonomous functional interaction between caspase-1 and iNOS in ALS mice. A detrimental feedback ioop appears to play a role between caspase-1-generated mature IL-1B and iNOSgenerated NO. 2) Caspase-1 and caspase-3 are regulated at the expression and activation levels. The regulation of additional caspases will be evaluated. 3) Evaluate a potential therapeutic role for minocycline in ALS. Investigate the mechanisms of minocycline-mediated neuroprotection. 4) Since neuroprotection conferred by caspase inhibition and Bcl-2 over expression occurs by acting at different stages of the cell death pathway, we hypothesize that the combination of caspase inhibition and Bcl-2 over expression will provide greater neuroprotection than either alone. A proper knowledge of the caspase-mediated pathways will aid in designing rational pharmacotherapy. Since the mechanisms of cell death in these devastating diseases appear to be shared, furthering the understanding of the mechanisms of neurodegeneration in ALS will likely result in benefits to other neurodegenerative diseases such as Huntington's, Parkinson's, and Alzheimer's disease. -

Principal Investigator: GERHARDT, GREG A
Grant Number: 3P50NS039787-05S1
Title: RESTORATION OF DOPAMINE FUNCTION IN PARKINSON'S DISEASE

Abstract: Unavailable

Principal Investigator: GIBSON, ALAN R
Grant Number: 1R01NS044592-01A2
Title: Influence of the Basal Ganglia on Cerebellar Action

Abstract: Diseases affecting the basal ganglia produce a variety of movement deficits, and these deficits are often totally disabling. Parkinson's disease, which affects about 1.5 million Americans, is a basal gangliar disease that leads to tremor, decreased spontaneous movement and slowness of voluntary movement. Drug treatment of Parkinson's disease with L-DOPA is only partially effective in relieving the motor symptoms of the disease, and prolonged drug treatment leads to severe side effects such as uncontrollable involuntary movements. Deep brain stimulation at specific sites in the basal ganglia can provide effective relief of Parkinson symptoms. Neither drug treatment nor deep brain stimulation restores damaged neural circuitry in the basal ganglia. Therefore, it is likely that these therapies prevent abnormal basal gangliar output from disrupting processing in other structures related to movement control. One major neural structure related to movement control is the cerebellum, but there are no direct connections between the cerebellum and the basal ganglia. We have discovered that disrupting activity in the cat red nucleus, which connects cerebellar output to the spinal cord, can produce motor symptoms that are strikingly similar to those of Parkinson's disease. The general hypothesis underlying this proposal is that motor deficits produced by basal gangliar disease are mediated by pathways that allow basal gangliar output to disturb processing in structures related to the cerebellum. Specifically, we hypothesize that basal gangliar output from the cat entopeduncular nucleus affects activity of cells in zona incerta, which affects activity of cells in the red nucleus. Our experiments will: 1. Identify regions in the related nuclei that contain cells related to forelimb movement. 2. Determine how these forelimb regions affect movement with activation and inactivation by injection of receptor antagonists. 3. Develop an acute and chronic cat model of basal gangliar disease to test critical aspects of the hypothesis. 4. Identify additional brainstem pathways that allow basal gangliar output to influence cerebellar circuits. The results will provide a deeper understanding of how the basal ganglia and cerebellum interact to control limb movements and will lead to new approaches for the treatment of movement disorders.-

Principal Investigator: GIBSON, ALAN R
Grant Number: 3R01NS044592-01A2S1
Title: Influence of the Basal Ganglia on Cerebellar Action

Abstract: Unavailable

Principal Investigator: Goldstein, David
Grant Number: 5Z01NS002979-06
Title: Clinical Neurocardiology: Catecholamine Systems In Stress And Disease

Abstract: Unavailable

Principal Investigator: GROSSMAN, MURRAY
Grant Number: 5R01NS035867-07
Title: Cognitive Impairments in Parkinson's Disease and Aging

Abstract: We seek converging evidence from cognitive studies of non-demented patients with Parkinson's disease (PD), electrocortical event-related potentials (CEPs), and functional magnetic resonance imaging (fMRI) to test our interactive neurocognitive model of core cognitive processes and executive resources in comprehension. Specific Aim 1 manipulates executive resources (working memory, strategic planning, inhibitory control) in ambiguous sentences. PD patients' impaired sentence comprehension will be related to limitations in specific executive resources. Resource-related slowing of CEPs will be seen in PD for the same material. fMRI in young subjects with this material will recruit interactive neural networks for sentence processing: left ventral inferior frontal cortex (vIFC) and left posterolateral temporal cortex (PLTC) for core language processes, and specific cognitive resources in left dorsal IFC (dIFC), prefrontal cortex, striatum, and right PLTC. To compensate for age- and disease-related resource limitations, healthy seniors and PD patients will up-regulate resource-related networks, but we expect no change in the core sentence processing network. Specific Aim 2 tests a material-neutral deficit for rules that depends on implicit memory. We examine regular and irregular morphology in verbs and nouns, and assess non-linguistic concept acquisition mediated by implicit- or rule-based learning. PD patients will show a material-specific deficit for rules in verbs. fMRI in young subjects will recruit left vIFC only for regular verb morphology, and dIFC for decision-making resources. d]FC will be up-regulated in aging and PD. Specific Aim 3 assesses the generalizeability of our model to prosody comprehension. PD patients judge acoustically simple and complex prosody stimuli at baseline and during a secondary task. Restricted resources will limit PD patients' comprehension of complex prosody. fMRI in young subjects will recruit orbital frontal and dIFC only for complex prosody, and dIFC will be up-regulated in aging and PD. Our data support a componential neurocognitive architecture consisting of dynamically interactive networks modified to process sentences depending on available resources and relative demand. -

Principal Investigator: HALVERSON, ROBYN A
Grant Number: 5F31NS043053-03
Title: Transglutaminase Cross Linking of Tau in FTDP17

Abstract: Tauopathies are a group of neurodegenerative disorders in which filamentous tau aggregates are a hallmark pathological lesion. The recent discovery of tau mutations in FTDP-17 demonstrates that tau dysfunction can directly cause neurodegeneration. However, the mechanism underlying the formation of neurofibrillary tangles (NFT) is unclear. Work from our laboratory demonstrates that transglutaminasecatalyzed cross-linking of tau is present in NFT in two tauopathies. Transglutaminase is a calciumdependant enzyme that covalently cross-links proteins rendering them insoluble, similar to the properties of NFT. It is not known what causes the increased cross-linking of tau in tauopathies. Preliminary data from our laboratory suggest that oxidative damage and elevation of intracellular calcium produce elevated transglutaminase-catalyzed cross-links in tau. We hypothesize that oxidative stress and disruption of calcium homeostasis may contribute to the transglutaminase-catalyzed cross-linking of tau and neurofibrillary pathology of tauopathies. Our first aim is to demonstrate that transglutaminase-catalyzed cross-links are present in tau from FTDP-17 patients and mice expressing VFDP-17 associated tau mutations. The second and third aim will utilize a cell culture model to assess the effects of oxidative stress and elevated intracellular calcium on the transglutaminse-catalyzed cross-linking of mutated tau. The proposed studies will help elucidate mechanism of NFT tangle formation, therefore providing potential avenues for therapeutic intervention in a broad range of tauopathies.-

Principal Investigator: HEIDENREICH, KIM A
Grant Number: 5R01NS038619-05
Title: IMPROVING SURVIVAL OF TRANSPLANTED DOPAMINE NEURONS

Abstract: Fetal dopamine neurons are implanted into the brains of Parkinson's disease patients in an effort to replace lost neurons and restore dopamine levels. Death of 95% of the transplanted neurons limits the usefulness of this experimental therapy. Transplanted dopamine neurons die in part by apoptosis (programmed cell death) and to a lesser extent by necrosis. This finding opens the exciting possibility that signaling pathways leading to the initiation and execution of the death program can be blocked while signaling pathways that protect against apoptosis can be activated to reduce neuronal death. In vitro experiments have shown that p38 mitogen-activated protein (MAP) kinase is activated during apoptosis in primary neurons and that a specific inhibitor of p38 MAP kinase rescues neurons from apoptotic cell death. Since apoptosis occurs in neural grafts during transplantation, we hypothesize that specific inhibitors of p38 MAP kinase will block apoptosis of fetal dopaminergic neurons transplanted into the striatum of Parkinsonian rats resulting in increased neuronal survival, better reinnervation of the striatum, and improved motor behavior. IGF-1, bFGF, insulin and GDNF also rescue cultured dopaminergic neurons from apoptosis. Based on studies in other types of neurons, these growth factors are thought to work by either stimulating antiapoptotic pathways, (i.e., IGF-1 stimulation of Akt) or inhibiting proapoptotic pathways (i.e., insulin inhibition of p38 MAP kinase). Since there are multiple pathways regulating apoptosis, we predict that combination of growth factors and inhibitors of p38 MAP kinase will provide additive protection of the transplanted grafts against apoptosis. Moreover, by further delineating the signaling pathways that mediate the antiapoptotic effects of the above growth factors, new cellular targets for therapy can be defined. The specific aims of the proposal are to: #1. Determine if specific inhibitors of p38 MAP kinase improve transplantation of fetal dopaminergic neurons. #2. Determine if the combination of p38 MAP kinase inhibitor and growth factors provides additive protection against apoptosis in transplanted tissue grafts. #3. Define the signaling pathways by which IGF-1, bFGF, insulin and GDNF promote survival of dopaminergic cells. Results from these studies will provide a basis for translating the use of p38 inhibitors and growth factors in transplantation to human studies and will provide new cellular targets that can be manipulated to prevent or arrest neuronal apoptosis. -

Principal Investigator: HOLROYD, SUZANNE
Grant Number: 5R01NS045008-02
Title: Parkinsons disease:Visual dysfunction and hallucinations

Abstract: The purpose of this three year grant is to examine the relationship between visual hallucinations and visual system abnormality in Parkinson's disease. Visual hallucinations are common symptoms and frequent causes of morbidity in Parkinson's disease, yet little is known about their etiology. Increasing evidence suggests that hallucinations in Parkinson's disease are not simply a medication effect, but are associated with the underlying disease process. Specificallly, evidence exists that suggest visual hallucinations in Parkinson's disease may be related to known visual system dysfunction in Parkinson's disease. In this study, thirty Parkinson's disease patients with visual hallucinations will be matched to thirty Parkinson's disease patients without visual hallucinations. They will be examined on neuropsychological tests assessing visual cognitive function, and will undergo visual evoked potentials. A subset of these patients (20 matched pairs) will also undergo functional magnetic resonance imaging (fMRI) to assess visual cortex function. It is hypothesized that Parkinson's disease patients with visual hallucinations will have greater evidence of visual system abnormality. Specifically they will demonstrate greater deficits of visual-cognitive function, greater latency on visual evoked potential and differences in activation of visual cortical regions on functional magnetic resonance imaging (fMRI) than those without visual hallucinations. It is hypothesized that these results will support a proposed biologic model of VH in PD regarding the role of dopamine abnormality in both the retina and basal ganglia that effect the regulation of function of visual cortex. The results of this study will increase knowledge regarding the neural mechanisms of visual hallucinations in Parkinson's disease and knowledge of visual system abnormality in Parkinson's disease. The results may also increase our understanding of visual hallucinations in other disorders. Conceivably, such knowledge could lead to strategies to prevent, minimize or treat such symptoms.-

Principal Investigator: HORVATH, TAMAS L
Grant Number: 5R01NS041725-03
Title: Uncoupling Protein 2 Promotes Neuronal Survival

Abstract: We have identified the existence of mitochondrial uncoupling protein 2 (UCP2) in homeostatic circuits of healthy rodents and non-human primates. We also showed that ectopic expression of this uncoupling protein is induced in different models of neurodegeneration, including models of Parkinson's disease, hypoxia, epilepsy or trauma-induced brain injury. The expression of UCP2 in these experiments was associated with subpopulations of neurons and microglial cells at the site of the degenerative processes and predicted cells with the longest survival after the initial insult. In our preliminary studies, UCP2 overexpressing animals had diminished levels of free radical production in the brain and responded to transection of the entorhinal pathway with suppressed caspase 3 activation. We hypothesize that the induction of UCP2 in neurons and glial cells during pathological neurodegeneration is an attempt to protect and rescue injured neurons. Three Specific Aims are proposed to test this hypothesis: Specific Aim 1 To determine the role of the UCP2 gene product in intracellular calcium homeostasis and protection of cells in vitro by studying PC12 cells and primary cultures of retinal ganglion cells with and without UCP2 transfection and primary cultures of retinal ganglion cells taken from UCP2 transgenic, UCP2 knockout and wild type mice. The effects of oxygen and glucose deprivation and glutamate agonists will be assessed on cell death patterns and intracellular calcium metabolism in these cultures. Specific Aim 2 To determine the pattern of neurodegeneration, mitochondrial uncoupling activity, cytokine and ATP production in the brains of UCP2 knockout mice, UCP2 overexpressing transgenic mice and wild type mice undergoing hypoxia-, seizure- or 1-methyl-4-phenyl- 1,2,5,6 tetrahydropyridine (MPTP)-induced neurodegeneration. Specific Aim 3 To assess the effects on phenotype development of superoxide dismutase 2 knockout animals that are crossbred with either UCP2 knockout or UCP2 overexpressing mice. In these experiments, we will follow the phenotypic alterations by assessing neuronal loss, level of mitochondrial uncoupling activity, cytokine, free radical and ATP production and intracellular calcium levels using morphological, biochemical and molecular biological approaches. The results of the proposed studies will shed light on a novel mitochondrial mechanism that plays critical roles in the suppression of neurodegeneration regardless of the initial cause of disease. This will furnish one common target for the development of drugs against a variety of neurodegenerative pathologies, including those associated with hypoxia, epilepsy, Parkinson's, Alzheimer's and Huntington's Disease. -

Principal Investigator: HUTSON, CHE B
Grant Number: 1F31NS051163-01
Title: The Role of Inflammation in Parkinson's Disease

Abstract: Parkinson's disease (PD) is a neurological disorder characterized by the degeneration of nigrostriatal dopaminergic neurons. The cause of this degeneration has yet to be fully understood. However, there is increasing evidence that PD is the result of a complex set of interactions encompassing genetic predisposition, the innate oxidative characteristics of the nigrostriatal dopaminergic pathway and inflammation. Less than 10% of PD cases are hereditary. A subset of which has been linked to two mutations in the alpha-synuclein gene. Our laboratory has obtained a mouse that over-expresses human alpha-synuclein under the control of the platelet-derived growth factor promoter. Using this mouse as a genetic model of PD, I plan to examine the inflammatory mechanisms leading to the loss of nigrostriatal dopaminergic neurons after exposure to the inflammagen lipopolysaccharide (LPS). I hypothesize that in the context of increased alpha-synuclein expression, inflammation is detrimental to dopaminergic neurons. Furthermore, I hypothesize that LPS mediated inflammation will result in the loss of dopaminergic cells in the substantia nigra of the alpha-synuclein over-expressing murine model of PD. -

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 5R01NS043309-03
Title: Using Stem Cells in Animal Models of Parkinson's Disease

Abstract: One promising new therapy for Parkinson's Disease (PD) involves the replacement of degenerated nigrostriatal neurons with those derived from transplanted fetal mesencephalic tissue. Although this approach has often yielded remarkable recovery of function in rats and monkeys, results in clinical trials with PD patients have been less consistent. At issue, is the relative inability to standardize a number of critical factors in human fetal transplants, including the age, type, number and integrity of cells being grafted. Consequently, finding more reliable sources of dopaminergic (DA) tissue for transplantation has become increasingly important. One direction has been to search for a line of readily available, well-characterized continually self-renewing stem or precursor cells that possess the capacity to differentiate, ideally spontaneously and with the need for little manipulation, into DA neurons, thus providing an inexhaustible and uniform source of replacement tissue. Towards this end, our preliminary findings demonstrate that grafts of embryonic mouse neural stem cells (NSCs) of the C17.2 cell can differentiate exclusively into neurons, which in a majority of cases, can express DA traits when cells are transplanted into the brain of a Parkinsonian rat. In addition, in preliminary studies using stem cells from adult human bone marrow (MSCs), we have found that nearly 100 percent of MSCs will convert into process- bearing, beta-tubulin III+ neuronal-like cells after only 1-2 hours of incubation with specific differentiation factors. If these cells also exhibit the same capacity as NSCs to respond to appropriate DA differentiation cues in vivo, patients could provide their own source of stem cells for autologous grafts in PD. Using NSC and MSC stem cell models and a multidisciplinary approach, our specific goals for this proposal are threefold: 1) Identify the conditions that promote the stable appearance of a postmitotic differentiated DA phenotype in stem cells grown in culture; 2) Identify those factors which promote the differentiation of a DA phenotype in transplanted stem cells and 3) Determine whether the DA phenotype in transplanted stem cells is stable and long lasting, and whether, it can produce functional recovery of motor deficits in a rat model of PD. The ultimate goal of this research program is a fuller understanding of the cellular and molecular processes regulating the differentiation of DA traits in stem cells and apply that knowledge to transplantation strategies for the treatment of Parkinson's Disease.-

Principal Investigator: IACOVITTI, LORRAINE M
Grant Number: 3R21NS043705-02S1
Title: Neural Stem Cells Grafts in Primate Models of Parkinsons

Abstract: Unavailable

Principal Investigator: ISACSON, OLE
Grant Number: 5R01NS041263-05
Title: ANTI-INFLAMMATORY THERAPIES NEUROTOXICALLY INDUCED PD

Abstract: A recent large Parkinson's Disease (PD) twin study indicates that environmental and toxic factors play major roles in causing typical PD (Tanner, et. al. JAMA, 1999). Interestingly, neuroinflammation seen in the caudate-putamen is a part of the pathophysiology (Brooks, 1999). The progressive decline of dopamine (DA) terminals seen in idiopathic PD can be closely modeled in Macaca fascicularis by low-dose exposure to the mitochondrial toxin, MPTP, over nine to fourteen months. The investigators demonstrated by PET imaging of DA terminal and MRS that such primates provide a physiological chart of degeneration and appearance of PD signs (Brownell, et. al., Nat. Med., 1998). This data profile enables the design of an experimental paradigm for realistically determining toxicity, neuroinflammation and neuroprotection in idiopathic PD. In this project using the PD primate model, the investigators now propose to examine neuroprotection of the dopaminergic system by anti-inflammatory agents. Based on several studies, they hypothesize that a cyclooxygenase (COX) 1 and 2 inhibitor (indomethacin [1-(4-chlorobenzoyl)-5-methoxy-2-methyl-1-H-indole-3-acetic acid]) can decrease inflammatory reactions caused by MPP+ toxicity and also reduce chronic neurodegenerative processes. In the non-human primate, a slow progressive lesion of the nigrostriatal dopaminergic system follows repeated MPTP treatment. Using PET scanning with a receptor ligand for the peripheral benzodiazepine receptor site (11-C-PK1 1195), preliminary experiments indicate that they can visualize the neuroinflammatory reactions during CNS DA degeneration (as determined by 11-C-CFT). These measurements will be combined with MRI and MRS studies of lactate and choline as in vivo biomarkers for the glial inflammatory and toxic responses of the nigrostriatal system. As a therapy, during and after neurotoxic exposure to MPTP, the investigators will treat the PD primates with a COX I and 2 inhibitor to evaluate anti- inflammatory prevention of onset and continued degeneration. Protection of the dopaminergic system by anti-inflammatory agents would be of tremendous therapeutic value for PD. -

Principal Investigator: ISACSON, OLE
Grant Number: 3P50NS039793-05S1
Title: NOVEL THERAPEUTIC APPROACHES FOR PARKINSON'S DISEASE

Abstract: Unavailable

Principal Investigator: Jacobs, JESSE V
Grant Number: 1F31NS048800-01
Title: rTMS over premotor cortices during stepping in PD

Abstract: This project will explore the role of the Supplementary Motor Area (SMA) and dorsal Premotor cortex (dPMC) in the postural preparation and execution of voluntary stepping with and without visual targets in healthy and Parkinson's disease (PD) human subjects. The excitability of these cortical regions will be 'temporarily depressed by repetitive transcranial magnetic stimulation (rTMS). Measures of step timing and placement, prepatory shifts in foot pressure, and electromyographical records of muscle activity will provide data regarding the effect of PD, visual condition, levodopa, and rTMS on step preparation and execution. Hypotheses: (1.) The SMA is important for movement preparation and postural coordination prior to a voluntary step, and the symptom of bradykinesia (slow step initiation and impaired preparatory shifts in foot pressure) in PD is associated with impaired activity in the SMA. (2.) The dPMC is important for integrating visual input to modify voluntary steps (step placement and trajectory), and PD subjects exhibit an increased dependence on vision and the activity of the dPMC to execute voluntary steps.-

Principal Investigator: JAKOWEC, MICHAEL W
Grant Number: 5R01NS044327-03
Title: Glutamate-dopamine plasticity in nigrostriatal injury

Abstract: The MPTP-lesioned mouse serves as an excellent model to study the mechanisms involved in the return of striatal dopamine after basal ganglia injury. The administration of MPTP to C57BL/6 mice leads to the destruction of nigrostriatal dopaminergic neurons and subsequent depletion of striatal dopamine. An advantage of MPTP-lesioning is that the degree of neuronal cell death can be titrated such that remaining dopaminergic neurons may act as a template for repair and recovery in response to the injury. Our hypothesis is that glutamate, acting through altered expression of the AMPA-subtype of receptor, activates the transcription factor phospho-CREB and leads to increased tyrosine hydroxylase expression and axonal sprouting in surviving nigrostriatal dopaminergic neurons. This research proposal is designed to define changes that take place after MPTP injury in the expression of AMPA receptors (including their phosphorylated state), the transcription factor CREB, dopamine receptors (Dl, D2, and D3), and the growth-associated protein GAP-43. The effect of blocking glutamate neurotransmission with the AMPA receptor antagonist GYKI-52466 on these parameters will be determined. The molecular tools of immunocytochemistry, western immunoblotting, in situ hybridization, and anterograde labeling will be used to define the mechanisms involved in the return of striatal dopamine. The long-term goal of these studies is to elucidate features of plasticity following injury to the brain and to identify new therapeutic interventions for the treatment of neurodegenerative diseases including Parkinson's disease, Alzheimer's disease, and aging. -

Principal Investigator: KANG, UN Jung
Grant Number: 5R01NS043286-02
Title: The neuroprotective effect of tetrahydrobiopterin

Abstract: While multiple etiologies are likely to account for Parkinson's disease (PD), the core pathogenic feature is degeneration of dopaminergic neurons, particularly those in the substantia nigra pars compacta (SNpc), with shared common final pathways involving oxidative damage, mitochondrial dysfunction, or both. Therefore, one may hypothesize that dopaminergic neurons in the SNpc are selectively vulnerable to oxidative stresses and/or mitochondrial disruption and understanding the mechanism of this selectivity may reveal the pathogenesis. However, our data show that ventral mesencephalic dopaminergic neurons in culture have an enhanced antioxidant capacity, as they are better able to resist oxidative stresses such as glutathione depletion and peroxide treatment than nondopaminergic neurons. In addition, their enhanced antioxidant capacity is reflected in lower reactive oxygen species (ROS) and higher reduced glutathione levels than nondopaminergic neurons. We hypothesize that an enhanced antioxidant capacity is essential for the survival of dopaminergic neurons that may be subjected to increased oxidative stress exerted by dopamine and its metabolites. We postulate that disruption of this innate antioxidant capacity makes them vulnerable to additional environmental insults and thereby leads to selective degeneration. We noted that the enhanced antioxidant capacity in ventral mesencephalic dopaminergic neurons is due to tetrahydrobiopterin (BH4), which is the cofactor for tyrosine hydroxylase, the enzyme producing dopamine, but also lowers superoxide levels, partly be direct scavenging effect and modulates mitochondrial function. First, We will study the effect of BH4 on mitochondrial bioenergetics and function including initiation of death pathways. Second, we will examine the role of BH4 on NO and superoxide generation and in modulating other endogenous antioxidant systems. Third, the neuroprotective function of BH4 against PD models such as 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine, rotenone toxicity, and glutathione depletion will be tested in vivo and in organotypic slice cultures, using hph-1 mice that are deficient in BH4, production.-

Principal Investigator: Kanthasamy, Anumantha
Grant Number: 5R01NS045133-02
Title: CASPASES, MITOCHONDRIAL FUNCTION AND PARKINSON'S DISEASE

Abstract: Parkinson's disease (PD) is a major neurodegenerative disorder affecting approximately 2% of the population over age 50, and the number of annual PD cases continues to rise along with the median age of the population. As the population in our society ages, we face the regrettable reality that effective medical treatment strategies for major chronic neurodegenerative disorders, including Parkinson's disease, are lacking. Determining the mechanisms of etiopathogenesis and selective nigrostriatal degeneration in PD is a formidable challenge. Emerging epidemiological and case control studies suggest that environmental factors, especially pesticides, are dominant risk factors in the etiology of sporadic, geriatric-onset Parkinson's disease. In this proposal, our preliminary data reveal that dopaminergic cells are susceptible to Dieldrin (a potential environmental risk factor for development of PD) -induced apoptosis, in which oxidative stress plays a causal role. We have also uncovered a novel apoptotic pathway involving caspase-3 dependent proteolytic cleavage of protein kinase Cdelta (PKCdelta) that not only mediates apoptosis in dopaminergic cells, but also influences key cellular events such as amplification of the apoptotic cascade through positive feedback activation and hyperphosphorylation of alpha-synuclein. We will extend our preliminary findings by pursuing the following Specific Aims: (I) characterize mitochondrial dysfunction and the subsequent activation sequence of key proapoptotic factors during dieldrin-induced oxidative stimulation in the mesencephalic dopaminergic cell model of Parkinson's disease, (ii) establish the proapoptotic function of caspase-3 dependent proteolytic activation of PKC5 in Dieldrin induced dopaminergic degeneration and to further investigate mechanisms underlying positive feedback amplification of the apoptotic signaling cascade by PKCdelta, (iii) obtain evidence to support the hypothesis that proteolytically activated PKCdelta hyperphosphorylates alpha-synuclein and thereby promotes protein aggregation, (iv) examine whether chronic exposure to Dieldrin in animal models induces caspase-3 dependent proteolytic cleavage of PKCdelta, alpha-synuclein aggregation, Lewy body formation and apoptotic cell death of dopaminergic neurons in the substantia nigra, and finally, (v) confirm the involvement of PKCdelta in nigral dopaminergic degeneration by using PKCdelta knockout animals and by targeted over-expression of PKCdelta and alpha-synuclein using a lentiviral delivery system in animal models. Together, results from the proposed systematic investigation will demonstrate the involvement of mitochondrial dysfunction, oxidative stress, apoptosis and

Principal Investigator: KAPLITT, MICHAEL G
Grant Number: 1K08NS044978-01A2
Title: PTEN Anti-Oncogene: Neuronal Function and Toxicity

Abstract: The PTEN anti-oncogene is among the most frequently mutated genes in malignant brain tumors. Normally, PTEN is a lipid phosphatase which blocks malignant phenotypes primarily by inhibiting the PI3 Kinase/AKT pathways, but PTEN can also act as a protein phosphatase. PTEN is expressed in brain late in development, and neuronal expression continues throughout adult life. Although loss of PTEN can cause neuronal hyperplasia, little is known about the role of PTEN in neuronal development or in normal neurons. Pathways influenced by PTEN suggest that this anti-oncogene may increase neuronal sensitivity to toxicity and/or degenerative processes, which is supported by our preliminary data. This proposal will first determine whether PTEN can modulate sensitivity of cultured neuron-like cells to toxins used in models of Alzheimer's disease and Parkinson's disease. While studying this hypothesis, we have unexpectedly found that PTEN blocks NGF signaling in PC12 cells, and this appears to be at least partially due to inhibition of expression of trkA and p75 NGF receptors at the protein and mRNA levels. DNA microarray then revealed that PTEN can inhibit expression of several genes, including tyrosine hydroxylase and GTP cyclohydrolase 1. Since this may also have implications for neuronal function and for Parkinson's disease, the second Aim of this proposal will also explore the mechanism by which PTEN inhibits expression of these genes. The final Aim of this proposal will explore the effect of age and neurotoxins used in models of neurodegenerative disorders on PTEN levels and function to determine the biological relevance of data generated from the first two Aims. These studies and my development as an independent clinical scientist will be significantly advanced by Dr. M. Flint Beal, who will serve as my sponsor and who is a leading expert in neuronal degeneration in PD and AD. Additional mentoring by Dr. Eric Holland, a leading expert on anti-oncogene signal transduction, will also add significantly to my scientific growth and will also help me to realize many of the Specific Aims of this proposal. The environment at Cornell and the strong support of my institution will permit me to focus upon these studies with minimal distractions. My scientific background is substantial, and this will facilitate realization of the goal of this project. This plan outlined in this award will, however, enhance previously underserved aspects of my education while focusing on an important scientific question, in order to promote a successful transition to scientific independence.-

Principal Investigator: KIM, KWANG S
Grant Number: 5R21NS044439-02
Title: DA-specific gene discovery and promoter engineering

Abstract: Gene therapy techniques need substantial development to provide therapeutic possibilities for treating neurological disorders such as Parkinson's disease (PD). Based on molecular control mechanisms of noradrenergic neuron-specific gene regulation, we recently devised a gene delivery system that can efficiently target transgene expression to noradrenergic neurons in a cell-specific manner. Our long-term goal is to establish gene therapy system(s) that will drive efficient transgene expression in a dopamine (DA) neuron-specific fashion based on discovery and characterization of DA-specific genes. Toward this end, we propose to identify and isolate genes that are selectively expressed in the DA mid-brain area by analyzing gene expression profiles using the most comprehensive cDNA microarrays such as the augmented NIA 16K chip and augmented RIKEN 16 K chip. Because these chips do not cover the whole genome yet, we will also identify novel DA-specific genes by the PCR-based subtractive hybridization techniques. Expression patterns of putative DA-specific genes will be tested by semi-quantitative RT-PCR using independently isolated mRNAs, and will be confirmed by in situ hybridization. Among the isolated DA-specific genes, we will first focus on putative DNA-binding transcription factors. The consensus binding sites for these putative transcription factors will be defined and their potential promoter function will be tested by cotransfection assays using cell line systems. On the basis of the mechanism of action of the novel DA-specific transcription factor(s), synthetic promoters will be developed and optimized. The optimized synthetic promoter will be subcloned in front of the reporter lacZ gene in the context of the self-inactivated lenti viral vectors. Cell type-specific expression of the reporter gene will be examined using both in vitro mesencephalic primary neuronal cultures as well as in different rat brain areas following stereotactic injection. At the later stage of this proposal, we will plan to use our developed promoter system(s) to deliver therapeutic genes (e.g., GDNF and Bcl 2) to the DA neurons and will test whether they can efficiently ameliorate behavioral symptoms in animal models of PD. The proposed research will identify and isolate genes that are selectively expressed in the mid-brain DA area on a genome-wide scale and will characterize their transcriptional regulation. Based on these mechanisms, we will devise novel and innovative DA-specific promoter systems and test them using in vitro and in vivo systems. In combination with safe viral vectors, our developed gene delivery systems can be translated clinically into gene therapy approaches for PD and other neurological disorders, in which DA

Principal Investigator: KONRADI, CHRISTINE
Grant Number: 1R01NS048235-01
Title: Levodopa dyskinesia and striatal neuroplasticity

Abstract: Parkinson's disease (PD) is a brain disorder caused by progressive loss of the brain chemical dopamine. Patients with Parkinson's disease are treated with levodopa (L-DOPA), a precursor of dopamine. However, L-DOPA therapy has disabling side effects. Most patients on L-DOPA treatment are eventually afflicted with motor fluctuations and abnormal, involuntary movements known as dyskinesias. L-DOPA-induced dyskinesias can become more disabling than Parkinson's disease itself. In severe cases, neurosurgical lesioning of basal ganglia nuclei such as the thalamus, pallidum or subthalamic nucleus is needed to improve Parkinson's disease and to minimize L-DOPA dosage. The proposal is based on the hypothesis that L-DOPA treatment in Parkinson's disease, and L-DOPA-induced dyskinesia, are accompanied by unique patterns of gene expression in the putamen. By comparing the gene expression patterns of dyskinesia to non-dyskinesia, we may find the critical factors responsible for the development of dyskinesia, or responsible for preventing the development of dyskinesia. Specific therapies could then be devised that could be co-administered with L-DOPA to prevent dyskinesias. We propose to investigate the molecular systems that are altered in L-DOPA-induced dyskinesia, and to find the 'molecular signature' of dyskinesia. We will study gene expression patterns in the post mortem putamen in Parkinson's disease in response to L-DOPA treatment (PD; Specific Aim 1) and in response to L-DOPA-induced dyskinesia (Specific Aim 2), and compare it to a rat model of L-DOPA-induced dyskinesia (Specific Aim 3). The role of five candidate genes for the development of, or compensation for, dyskinesia will then be examined in the rat model (Specific Aim 4). In a gene array experiment we have already collected data from the rat model of dyskinesia and assembled lists of candidate genes from these data. The lists of genes will be cross-referenced with the findings in the human putamen to determine five most likely candidates to be tested in the rat model. Hypothesis testing will be combined with computer programs that can find interesting new, unanticipated patterns of gene regulation, and help to formulate new hypotheses. The post mortem samples provide us with direct access to the human condition, while the animal model provides us with an experimental system that can be tightly controlled and that permits functional analyses and hypothesis-testing. Together they can lead the way toward new treatments for dyskinesia. -

Principal Investigator: KONTOPOULOS, EIRENE
Grant Number: 1F31NS049869-01
Title: Mechanisms of Neurotoxicity in Parkinson's Disease

Abstract: Our long-term objective is to elucidate the underlying mechanisms of neuronal death in Parkinson's disease (PD). The identification of several genes exhibiting linkage to PD has not yet led to the understanding of how their protein products bring about cell death. Though in vitro studies have been instrumental in identifying potential mechanisms of neurodegeneration, their findings need to be corroborated in vivo. I propose to utilize Drosophila genetics to investigate putative protein interactions among three PD-linked genes: synphilin-1, alpha-synuclein, and parkin. My primary strategy will be to investigate the inherent toxicity of synphilin-1 and its PD-associated mutation, R621C. Furthermore, genetic interactions between both forms of synphilin and either alpha-synuclein or parkin will be examined. These efforts will culminate in the investigation of genetic interactions among synphilin-1, alpha-synuclein, and parkin. -

Principal Investigator: KORDOWER, JEFFREY H
Grant Number: 5R01NS043290-03
Title: DYSKINESIAS IN LENTI-GDNF TREATED PARKINSONIAN MONKEYS

Abstract: Fetal nigral grafts can cause "runaway" dyskinesias in patients with Parkinson's disease (PD;Freed et al., 2001). These dyskinesias are severe, debilitating and strongly indicate that 1) novel dopaminergic surgical therapeutic strategy planned for clinical trials need to be tested preclinically for their effects upon dyskinesias and 2) the mechanisms underlying these dyskinesias need to be elucidated. We have recently demonstrated that lentiviral gene delivery of glial cell-derived neurotrophic factor (GDNF) potently prevents motor dysfunction and prevents nigrostriatal degeneration in nonhuman primate models of PD (Kordower et al., 2000). Prior to initiating clinical trials with lenti-GDNF, it effects upon dyskinesias need to be evaluated in parkinsonian monkeys. Freed, Fahn and coworkers (2001) have hypothesized that grafted-mediated dyskinesias result from graft overgrowth. However, their own PET and post-mortem data, as well as the data from others (Kordower et al., 1995, Lee et al 1999), do not support this view. We propose an alternative hypothesis that these dyskinesias result from local "hot spots" of hyperdopaminergic function interacting with the levodopa primed brain. We plan to test this hypothesis by comparing gene therapies that induce either a) widespread or b) local hyperdopaminergic function upon dopa-induced dyskinesias and the role of dopa priming. This application will have three Specific Aims. Specific Aim 1 will test the hypothesis that lenti-GDNF treatment to non-levodopa primed MPTP-treated monkeys will prevent, or diminish the intensity of dyskinesias when they are later treated with levodopa. Specific Aim 2 will test the hypothesis that lenti-GDNF will diminish the dyskinesia profile in dyskinesic MPTP-treated monkeys previously primed with levodopa. Specific Aim 3 will test the hypothesis that "hot- spot" hyperdopaminergic function, but not homogenous hyperdopaminergic innervation, will enhance the dyskinesia profile of parkinsonian monkeys and that elimination of GDNF will reverse the functional and dyskinesic effects established previously by this trophic factor. The study of dyskinesias has become a compelling area of PD research. Exciting therapeutic strategies such as gene therapy need to be evaluated for their effects on dyskinesias so that they are both safe and effective. This application will determine whether potent dopaminergic gene therapies influence dyskinesias in the best animal model of PD.-

Principal Investigator: KOTZBAUER, PAUL T
Grant Number: 1K08NS048924-01
Title: Neurodegenerative consequences of PanK2 mutations

Abstract: The candidate is an M.D./Ph.D neurologist who is currently a trainee in the Center for Neurodegenerative Disease Research. His goal is to develop additional research skills and experience needed to become an independent clinician scientist working to understand the pathogenesis of neurodegenerative diseases. The proposed research project focuses on neurodegeneration with brain iron accumulation (NBIA), which causes progressive impairment of speech, movement and cognition. At the neuropathological level, NBIA is characterized by iron accumulation, inclusion formation, signs of oxidative stress, and death of multiple neuronal populations. These features are also seen to varying degrees in other neurodegenerative diseases, including Parkinson's disease and Alzheimer's disease. Mutations in the gene for pantothenate kinase 2 (PanK2) were recently identified in a subset of NBIA cases. The PanK2 gene encodes an enzyme involved in coenzyme A (CoA) synthesis, a critical pathway linked to a number of cellular processes, including fatty acid synthesis, energy production, and possibly, synthesis of anti-oxidant molecules. The long term objectives of this project are to understand how PanK2 mutations lead to iron accumulation, oxidative stress, inclusion formation, and neuronal death. The proteolytic processing, mitochondrial localization and in vitro catalytic properties will by characterized for mutant Pank2 proteins and compared to the wild type human PanK2 protein. Cell culture systems will be established in which PanK2 expression is eliminated and in which wild type or mutant PanK2 proteins are over-expressed. Mice that lack PanK2 expression will also be generated. Cell lines and mice lacking PanK2 expression will be examined for changes in levels of biochemical intermediates hypothesized to be dependent on PanK2 function. Finally, neuronal and non-neuronal cells lacking PanK2 will be examined for signs of increased oxidative stress, susceptibility to oxidative injury, cellular and mitochondrial import of radio labeled iron, and inclusion formation.-

Principal Investigator: KRAMER, HELMUT J
Grant Number: 5R01NS043406-03
Title: Hook proteins in membrane trafficking & neurogeneration

Abstract: Neurodegenerative diseases such as Huntington's disease, amyotrophic lateral sclerosis or Parkinson's disease share one common feature, the slow accumulation of misfolded proteins. As misfolded proteins accumulate in neurons they are not evenly distributed. Instead, they are concentrated in inclusion bodies. How these inclusion bodies are linked to the progression of neurodegenerative diseases is not well understood. One class of inclusion bodies, aggresomes, are formed at the microtubule organizing center in an active process that requires microtubule-based transport. We recently discovered that the active concentration of misfolded proteins in aggresomes involves the Hook2 protein. Hook proteins constitute a family of coiled-coil proteins which bind to microtubules and affect the organization of different organelles in mammalian cells and in Drosophila. In this grant, we will combine genetic approaches in Drosophila, cell biological approaches in mammalian tissue culture cells and biochemical experiments in-vitro to characterize shared functions of Hook proteins, as well as the specific role of Hook2 in the cellular trafficking of misfolded proteins. In Spec. Aim 1, we will determine the relevance of microtubule binding of Hook proteins using a combination of biochemical approaches in vitro and genetic experiments in Drosophila. In this context we will also explore the potential interaction of Hook proteins with the complex between cytoplasmic Dynein and Dynactin. In Spec. Aim 2, we will characterize the binding of Hook proteins to different organelles and identify the receptors that mediate these interactions. In Spec. Aim 3, we will determine the role of Hook2 in the formation of aggresomes and the potential of using dominant-negative forms of Hook2 to manipulate the aggregation of different misfolded proteins. In Spec. Aim 4, we will determine the domains of Hook proteins responsible for their polarized distribution in neurons and the role of Hook proteins in establishing neuronal polarity in rat hippocampal neurons. -

Principal Investigator: KULAK, JENNIFER M
Grant Number: 5F32NS043079-04
Title: Changes in nicotinic receptors in parkinsonian animals

Abstract: As an initial approach, the research proposed in this application will identify specific subtypes of nicotinic acetyicholine receptor (nAChR) present in the nigrostriatal system which may be investigated for development of novel therapeutics for treatment of Parkinson's disease. Evidence that nAChRs may provide a therapeutic target for treatment include: (i) the demonstration of an inverse relationship between smoking and the incidence of PD; (ii) alleviation of the locomotor symptoms of PD with nAChR agonist administration; (iii) selective changes in expression of nAChR mRNAs in squirrel monkeys lesioned with the nigrostnatal toxin MPTP; and (iv) preliminary results indicating alterations in nAChR binding in parkinsonian non-human primates. Nicotinic receptor subtypes expressed in the nigrostriatal system will be investigated using 1251-epibatidine, 3H-cytisine, and '251-a-bungarotoxin autoradiography and competition with subtype-selective IIAChR ligands in the substantia mgra, caudate, and putamen of control and parkinsonian monkeys and the neurotransmitter makeup to which specific subunits colocalize will be determined by dual label in situ hybridization in the substantia nigra.-

Principal Investigator: LAI, CARY H
Grant Number: 5R21NS045690-02
Title: The RGS-9 mouse: inducible expression in the striatum

Abstract: Our ability to develop useful treatments for neurodegenerative diseases such as Parkinson's and Huntington's disease is hampered by our lack of understanding of the etiology of these disorders. One way of addressing the problem is to develop animal models of the disease and to use these to test hypotheses concerning the origin and progression of the neural degeneration. The ability to test these hypotheses and to create new models for these disorders would be greatly facilitated by the ability to generate mice that express a selected gene exclusively in the striatum. Here we propose to produce transgenic mice permitting the regulated expression of genes in a subset of striatal neurons. We propose to construct a transgenic mouse line ("the RGS-9 mouse") using the promoter and other regulatory elements for the gene encoding the regulator of G-protein signaling-9 (RGS-9) to drive the expression of the tetracycline transactivator. When mated to a distinct transgenic line containing a candidate gene whose transcription is regulated by the tetracycline operator, the resulting offspring should express the candidate gene in striatal neurons in the presence of the inducer, tetracycline. The availability of mice that inducibly express genes in striatal neurons should help neuroscientists tackle Parkinson's and Huntington's disease by facilitating the production of new models for these diseases. As additional genetic loci related to the etiology or progression of these diseases emerge from our improved understanding of the human genome, these genes or altered versions of these genes can be specifically re-inserted into striatal neurons to assess their effects. Use of the RGS-9 mouse would be superior to currently available systems, which express genes in larger collections of cells. Through these efforts, we hope to create a genetic tool that will accelerate the development of therapeutics for the patient with Parkinson's and Huntington's disease. -

Principal Investigator: LAI, CARY H
Grant Number: 5R21NS044941-02
Title: THE DOPAMINERGIC MOUSE

Abstract: Unavailable

Principal Investigator: LANGSTON, J W
Grant Number: 5R01NS034886-07
Title: MECHANISMS OF DOPA-DYSKINESIAS IN PARKINSONIAN MODELS

Abstract: This is the first competitive continuation of an ongoing NIH application to investigate dopa-dyskinesias in parkinsonian MPTP-lesioned monkeys. The long-term goal of this work is to elucidate the mechanisms underlying this devastating complication of chronic L-dopa therapy, which is a major barrier for the successful treatment of Parkinson's disease. During the course of our ongoing grant, we unexpectedly observed that normal animals also developed dopa-dyskinesias, in contrast to previous work which suggested that a nigrostriatal deficit is essential for this complication of L-dopa therapy. This new non-lesioned model of dopa-dyskinesias may provide insight concerning the etiology of these movement abnormalities because it allows us to investigate this phenomenon in a setting that is not confounded by an already damaged nigrostriatal system. By examining the biochemical changes caused by L-dopa in unlesioned as compared to MPTP-lesioned animals, we should be able to identify common molecular mechanisms that underlie the development of typical dyskinesias. In this competitive continuation, the behavioral, cellular and molecular mechanisms associated with L-dopa-dyskinesias will be studied. This will be approached by (1) testing the hypothesis that a compromised nigrostriatal dopamine reuptake system predisposes to dopa-dyskinesias. This will be studied by initiating a drug-induced impairment of dopamine reuptake in normal animals to determine if there is an enhanced susceptibility to dopa-dyskinesias. (2) We will also investigate the relative roles of dopamine receptor subtypes (D1, D2 and D3) in the genesis of dopa-dyskinesias by administration of receptor subtype specific agonists and antagonists. (3) Our third specific aim will involve experiments to determine the integrity of the nigrostriatal system in the different groups of monkeys with dopa-dyskinesias. (4) Lastly, we will study the molecular events in the basal ganglia which mediate the development of dopa-dyskinesias using different models of dyskinesias described above. This will include alterations in dopamine receptor-linked coupling mechanism (such as dopamine-stimulated 35SGTPgammaS binding, DARPP-32 phosphorylation and adenylate cyclase activity), changes in NMDA receptor number and phosphorylation, and alterations in PPE mRNA levels. The results of this work will advance our understanding of the molecular mechanism responsible for the debilitating dyskinetic movements which occur as a consequence of long-term L-dopa treatment in Parkinson's disease. -

Principal Investigator: LANSBURY, PETER T
Grant Number: 1R21NS047420-01A1
Title: High Throughout Assay to Probe UCH-L1 Ligase Inhibitors

Abstract: Parkinson's disease (PD) is characterized by the presence of Lewy bodies (the cytoplasmic neuronal inclusions) and the significant loss of dopaminergic neurons in the substantia nigra, a-synuclein was identified as one major fibril component of the Lewy bodies, thus linked the accumulation of this protein to the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of the pathogenesis of PD. Failure to regulate the concentration of a-synuclein, for example by dysfunction of degradation process, can also contribute to the build-up and consequently fibrillation of the protein. A gene, PARK5, has been linked to PD are involved in proteasomal degradation pathway and it is an ubiquitin C terminal hydrolase (UCH-L 1) that hydrolyzes C-terminal ester and amides of ubiquitin and is believed to play a key role in processing polyubiquitin and/or ubiquity lated proteolytic peptide. A rare mutation (193M) of UCH L 1 that yields a 50% reduction in its hydrolytic activity has been tentatively linked to a rare early onset form of PD, at the same time a polymorphism of the enzyme (S 18Y) was indicated to reduce the risk of PD. The assumption that each enzyme expresses a single enzymatic activity in vivo, however, is challenged by the linkage of UCH-L 1 to PD. UCH-L 1, especially those variants linked to higher susceptibility to PD, causes the accumulation of a-synuclein in cultured cells, an effect that cannot be explained by its recognized hydrolase activity. UCH-L1 exhibits a second, dimerization-dependent, ubiquityl ligase activity. The polymorphic variant of UCH-L1 that is associated with decreased PD risk (S 18Y) has reduced ligase activity, but comparable hydrolase activity as the wild-type enzyme. Thus the ligase activity, as welt as the hydrolase activity of UCH-L1 may play a role in proteasomal protein degradation, a critical process for molecules ("molecular probes") that can be used to perturb UCH-L1 ligase activity in cell culture and animal models of PD. This "chemical genetic" strategy is complementary to traditional genetic approaches (e.g., knockouts and trasngenics) for understanding protein function but has a distinct advantage in that the probes are potential lead compounds for the development of novel PD therapetutics. The program detailed below will seek probes with the following activities: (1) inhibitors ofUCH-L1 dimerization, (2) inhibitors of UCH-L1 ligase activity, and (3) repressors and activators of UCH-L1 expression. -

Principal Investigator: LANSBURY, PETER T
Grant Number: 3P50NS038375-05S1
Title: FAMILIAL PARKINSON'S DISEASE: CLUES TO PATHOGENESIS

Abstract: Unavailable

Principal Investigator: LAU, YUEN-SUM
Grant Number: 5R01NS047920-02
Title: Impact of Exercise on Parkinson's Disease Therapy

Abstract: Parkinson's disease (PD) is a slow, progressive, debilitative, neurodegenerative disease, which has no cure. The current pharmacological therapies only temporarily mask symptoms, but do not protect neurons from further degeneration. Furthermore, chemotherapeutic agents often cause severe adverse effects and reduce the effectiveness of treatment. Numerous clinical reports have suggested that endurance exercise can slow down disease progression, and add years of independent and quality life to PD patients, or even improve the delivery and efficacy of L-DOPA treatment. Exercise therapy, or in conjunction with drug therapy at early onset of disease state, have been highly advocated by recent clinical trials. The potential health benefit and neurological mechanisms of action for exercise on PD rehabilitation have not been rigorously tested in the laboratory animal models. This research is designed to elucidate the impact of endurance exercise training on nigrostriatal dopamine (DA) neuron plasticity using a slow, progressive, and neurodegenerative mouse model of PD developed and characterized by our laboratory. This model is established based on a regimen of chronic 1-Methyl-4-phenyl - 1,2,3,6-tetrahydropyridine (MPTP) injections co-administered with probenecid, a drug that inhibits the peripheral and neuronal clearance of MPTP and potentiates the neurotoxicity of MPTP. In this model, we observed a marked decrease of nigrostriatal DA function within one week after treatment and remained low for 6 months. The animal also shows a gradual loss of substantia nigra (SN) neurons, decline of motor activity, and an accumulation of c-synuclein-immunoreactive inclusions in the SN. We further present in the application our preliminary findings supporting the feasibility and potential neuromodulatory role of endurance exercise on enhancing nigrostriatal DA transmission and PD rehabilitation using this model. In this research, we will test the following hypotheses centered on the endurance exercise, when administered at an early stage in the parkinsonian (PK) mice, will 1) improve their mobility and physical rehabilitation, 2) improve the efficacy of L-DOPA, 3) produce these effects by mechanistically causing an elevation of BDNF expression, an increase in the differentiation of DA progenitor cells, and an enhanced DA transmission and plasticity in the nigrostriatal neurons. Findings from this research should provide new insight into the development of alternative therapeutic approaches for enhancing the conventional pharmacological treatment and rehabilitation of PD. Potential benefits for using such a synergistic approach in managing PD would likely reduce the risk of drug toxicity and lower the cost of health

Principal Investigator: LAURING, BRETT
Grant Number: 1R01NS043298-01A2
Title: Identification of Novel Alpha Synuclein Binding Protein

Abstract: Parkinson's Disease (PD) is the second most common neurodegenerative disease. As in many other neurodegenerative diseases, conformational alteration of a specific neuronal protein results in the accumulation of fibrillar amyloid inclusions, which in the case of Parkinson's disease, are termed Lewy Bodies (LBs). LBs have a fibrillar core with the fibrils being comprised primarily of a protein of unknown function called alpha-synuclein. Alpha-synuclein mutations cause autosomal dominant Parkinson's disease. Thus both human genetic and histologic evidence link synuclein to Parkinson's disease. Alpha-synuclein is a 140 aa protein which is 'natively unfolded' meaning that it has no identifiable secondary structure. However, in the presence of certain lipid membranes is can fold into a alpha helical conformation, and when incubated alone can fold into a beta-sheet rich conformation which allows it to form amyloid fibrils resembling those seen in Lewy bodies. Consistent with the hypothesis that alteration of synuclein conformation is linked to development of Parkinson's disease, purified mutant synuclein fibrillizes more rapidly than wild-type protein in vitro. Overexpression of synuclein as a transgene results in formation of Lewy body-like pathology in mice and flies. Synuclein expressed at endogenous levels rarely forms amyloid (only in PD patients), is not stably membrane-associated, and remains 'unfolded'. The discrepancy between the in vivo folding parameters and those observed in vitro leads us to hypothesize that synuclein-interacting molecules may regulate synuclein conformation, stabilize it in the 'unfolded' state, or regulate membrane binding. We therefore set up a novel photo-cross linking assay heretofore not used to study synuclein to identify synuclein binding proteins present in brain extracts and present at endogenous levels of expression to begin to determine how synuclein conformation is regulated. We have identified novel synuclein binders. We propose to develop a fluorescence resonance energy transfer assay capable of indicating synuclein conformation both in vivo and in vitro. That will allow for screening of proteins and synthetic agents capable of altering synuclein aggregation. These studies will enable us to define the range of proteins or agents to be further characterized in in vivo models of Parkinson's disease.-

Principal Investigator: LAWRENCE, MATTHEW S
Grant Number: 1R43NS048786-01
Title: Genomic markers of environmental toxins for Parkinsonism

Abstract: Parkinson's disease is a prevalent and devastating neurodegenerative condition of unknown etiology. One prominent hypothesis holds that the selective loss of the nigrostriatal dopaminergic neurons characteristic of the disease results from damage from environmental neurotoxins in genetically vulnerable individuals. Identifying such environmental contributors to Parkinson s pathogenesis represents a significant public health concern. This project aims to identify the in vivo gene expression changes that occur in the primate brain in response to environmental toxins that have been implicated in the production of Parkinson's and compare these changes with the selective neurotoxin, MPTP, and with the limited knowledge of genetic abnormalities in some Parkinson's patients. Because of the unique vulnerabilities of nonhuman primates and humans to dopamine neurotoxic agents, studies in primates are essential to uncover common genetic markers of toxicity and to reveal the potential toxicity of chemicals of unknown liability. The proposed Phase I studies will test the hypotheses that transcriptional changes that accompany and precede dopamine cell death can be identified using high density gene arrays and bioinformatics in the primate nigrostriatal system in vivo following MPTP exposure. Changes in mRNA initiation of regimen of 3 doses of MPTP over 36 hours that has been established to result in Parkinsonism. Expression changes will also be assessed 6 hours after the administration of a single dose. Changes in nigrostriatal dopamine concentrations and tyrosine hydroxylase immunohistochemistry will be assessed at all time points. Additionally neurobehavioral changes will be assessed in the 20-day animals. Together these data will allow a determination of the sequence of transcriptional changes that parallel or precede histological, biochemical and behavioral events, and allow an assessment of transcriptional events related to acute versus chronic toxicity, with confirmation by quantitative RT-PCR. Defining the chronological and dose dependent gene expression changes induced by MPTP may reveal a transcriptional profile that is predictive of nigrostriatal injury from this toxin. Phase II studies will address whether similar gene expression changes and neuronal injury are seen following exposure to environmentally prevalent compounds that are postulated to be risk factors for the development of Parkinson's disease, and to integrate the resulting transcriptional data into a toxicogenornic database and potentially customized microarrays which may be applied to the assessment of compounds for their possible health risk.-

Principal Investigator: LI, SENLIN
Grant Number: 1R01NS046004-01A1
Title: Macrophage Gene Therapy of Neurodegenerative Diseases

Abstract: Neurodegenerative diseases affect a large population of patients. Existing therapies are not satisfactory. Gene therapy holds promise, but focal delivery of DNA and the level of gene expression are challenging. Macrophages are recruited from bone marrow to most tissues of the body including the CNS, thus making them an attractive option for gene delivery. Galactosialidosis (GS) has been corrected by bone marrow-derived macrophages expressing human protective protein/cathepsin A (PPCA) transgene in a mouse model (PPCA-/-). However, correction in the CNS was incomplete due in part to weakness of the CSF-1R promoter used in the study. We have developed a series of super macrophage promoters (SMP) that are up to l00-fold stronger in vitro than the CSF-1R promoter. In models of the highly prevalent Parkinson's disease (PD), local delivery of glial cell line-derived neurotrophic factor (GDNF) has been found beneficial. We hypothesize that highly effective CNS delivery of GDNF can be achieved with the use of our super macrophage promoters and this will greatly ameliorate the pathologic changes and neurological defects in animal models of PD. To explore this hypothesis, our specific aims are: 1) To characterize these super macrophage promoters by transplantation of bone marrow stem cells transduced ex vivo with lentiviral vectors and in transgenic mice using EGFP (enhanced green fluorescent protein) as a reporter. Promoters with the greatest strength and tissue-specificity for macrophages will be used in the subsequent aims. 2) To ameliorate neurodegeneration in the MPTP (1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine) mouse model of Parkinson's disease by syngeneic transplantation of HSC transduced ex vivo with lentivectors expressing GDNF gene in macrophages/macroglia driven by the SMP. Bone marrow stem cells will be transduced ex vivo with GDNF expressing lentivirus and transplanted into lethally irradiated recipient mice. Four weeks after bone marrow transplantation, the recipient mice will be injected subcutaneously with MPTP. At selected time points post MPTP administration, PET scan and behavioral testing will be performed, and brain tissue will be examined for dopamine uptake and expression of tyrosine hydroxylase (TH). In the substantia nigra pars compacta (SN), dopaminergic neurons will be counted and cell apoptosis will be assessed by TUNEL staining and immunohistochemistry for active easpase-3. 3) To ameliorate neurodegeneration in the same way as in Aim 2, but GDNF expression will be controlled by a tetracycline-regulatable gene expression system. To evaluate the effects of macrophage/ super promoter-mediated delivery and expression of GDNF on degenerating

Principal Investigator: LING, ZAODUNG
Grant Number: 5R01NS045316-02
Title: Prenatal Endotoxin as a Model of Parkinson's Disease

Abstract: Unavailable

Principal Investigator: Meredith, Gloria
Grant Number: 5R01NS041799-05
Title: Synaptic Proteins, Trophic Factors and Neurodegeneration

Abstract: One of the most fundamental questions related to the progressive nature of neurodegeneration in human disease is how neurons die. Protecting nerve cells against morphological decline and death requires blocking intrinsic factors that inhibit neural repair. In the present proposal, we offer an innovative approach to study those factors that are active in Parkinson's disease (PD) in a new mouse model that shows synaptic loss and irreversible nigrostriatal degeneration. We propose to track changes of a key synaptic protein, a-synuclein, both in its native environment at presynaptic terminals and under neurotoxic conditions, when it becomes insoluble and accumulates. We will further correlate those changes with altered neurotrophic support. We have established an animal protocol by treating C57/bl mice with a combined regimen of 10 doses of probenecid at 250mg/kg and MPTP at 25mg/kg for 5 weeks. These mice show a slow, progressive loss of nigrostriatal dopaminergic function for at least 6 months, that mimics PD, with no signs of recovery. Three weeks after drug treatment, there is a significant reduction in the number of substantia nigra (SN) cells and dramatic changes in the subsynaptic distribution and density of a-synuclein-immunoreactive terminals. These changes could signal the beginning of a chain of events that leads to cell death. In this proposal, we will focus on the progressive deterioration of dopaminergic neurons in the SN and their inputs, and present three specific aims to be addressed through a series of hypotheses. Specifically, we plan to 1) ascertain the origin and neurochemical phenotype of synapses in the SN that contain a-synuclein and to establish whether MPTP + probenecid treatment leads to their degeneration; 2) determine, in the MPTP+P model, the temporal relationships between cell death and a-synuclein-positive synapses, decline in dopamine function and behavior; and 3) ascertain whether changes in a-synuclein expression and production are precipitated by altered neurotrophic support. The overall objective of our research is to understand the relationship between the synaptic protein, a-synuclein, neurotrophic support, especially brain-derived neurotrophic factor (BDNF) and their respective roles in the PD form of neurodegeneration. The findings of this research should shed light on target areas where neuroprotection strategies can be implemented. -

Principal Investigator: MORGAN, JAMES I
Grant Number: 5R01NS040361-04
Title: Mechanisms of Cell Death in the Nervous System

Abstract: Programmed cell death (PCD) is a strictly regulated process and its disruption results in myriad developmental deficits and pathological sequelae. PCD is especially critical in the mammalian nervous system where its perturbation results in aberrant neural development and contributes to many neural disorders. There has been much research into the role of caspases in cell death. However, there is growing evidence for the importance of caspase-independent cell killing in the mammalian nervous system and other tissues. It is difficult to identify the components of the latter pathway or establish its contribution to cell elimination in vivo as it is intimately interwoven with, and masked by, the ubiquitous caspase cascades. We have developed a paradigm that can circumventthis limitation. CED-4S is a pro-apoptotic protein from C. elegans that is lethal when expressed in Saccharomyces cerevisiae. CED-4S lethality in yeast shows physiological specificity as it is blocked by its natural antagonist, CED-9 and is not mimicked by its anti-apoptotic splice variant, CED-4L. However, CED-4S toxicity in yeast does not require a caspase. Given the high degree of structural conservation amongst components of the cell suicide machinery, we propose to use CED-4S lethality in yeast as a paradigm to isolate molecules involved in caspase-independent killing. Subsequently, we will identify the mammalian counterparts of these molecules and investigate their function in the vertebrate nervous system. Using a CED-4S suppresser screen, we isolated 2 yeast AAA-ATPases, Cdc48 and yAPO-1 that have homologs in higher eukaryotes that have been implicated in neuronal death. Cdc48 binds to CED-4 whereas yAPO-l does not. This suggests a scenario in which CED-4S complexes with Cdc48 and alters its function, thereby leading to death. yAPO-l may have a redundant function with Cdc48 or it may lie downstream in the caspase-independent death pathway. Based upon these findings, we will use yeast and mammalian models to characterize the caspase-independent death pathway and determine the role that these and other CED-4 suppressers play in neuronal death in mice. In Specific Aim 1, we will determine the composition and functional domains of CED-4-containing complexes in yeast. In Specific Aim 2, downstream targets of CED-4 will be identified in yeast using a CED-4S suppresser screen. In Specific Aim 3, we will determine the expression and function of the mammalian homologs of the CED-4 suppressers in developing and adult brain and assess their contribution to normal and pathological cell death in the nervous system. -

Principal Investigator: Mouradian, Maral
Grant Number: 5Z01NS002826-14
Title: Molecular Pathogenesis Of Cell Death In Neurodegenerative Diseases

Abstract: Unavailable

Principal Investigator: MURCIA, CRYSTAL L
Grant Number: 5F32NS043844-03
Title: The Role of the En-l Pathway in Parkinson's Disease

Abstract: Parkinson?s disease (PD) is a common progressive neurodegenerative disease affecting approximately 2 percent of the population over age 65 throughout the world. Evidence from studies of idiopathic PD suggests that it is a complex disease involving multiple genetic and environmental factors. We have developed a potential mouse model of idiopathic Parkinson?s disease in the Engrailed-i (En-i) knockout mouse, En aboutiM. Homozygous En about ih/d mice on the 129/Sv inbred strain display severe cerebellar hypoplasia and perinatal lethality. In contrast, on the C57B1/6J inbred strain, homozygous mice are viable and exhibit tremors and hesitant gate reminiscent of Parkinson?s disease. To further analyze the role of strain background on the En-i mutant phenotype we propose the following three specific aims: 1) further characterize the Parkinson?s disease phenotype of En-i deficient mice, 2) genetically map strain-specific modifier genes required to produce the PD phenotype in En-1hd mutant mice, and 3) analyze candidate modifier genes for strain-specific alterations that contribute to the PD phenotype. The discovery of new genes with a genetic link to PD will provide future targets for therapeutic intervention.-

Principal Investigator: Oldfield, Edward
Grant Number: 5Z01NS002813-15
Title: Drug Delivery Techniques

Abstract: Unavailable

Principal Investigator: Oldfield, Edward
Grant Number: 5Z01NS002854-13
Title: Pathophysiology Of Neurosurgical Disorders

Abstract: Unavailable

Principal Investigator: O'MALLEY, KAREN L
Grant Number: 2R01NS039084-05A1
Title: Mechanisms of Neuronal Death in Parkinson's Disease

Abstract: Oxidative stress is a major factor in Parkinson's Disease (PD). Dopamine (DA) itself is easily oxidized to quinone derivatives and reactive oxygen species (ROS) that impair energy metabolism and form adducts with proteins such as upsilon-synuclein. Because pharmacological depletion of DA in animal models is confounded by non-specific peripheral and central nervous system effects, the role of DA oxidation in nigral cell death has been previously impossible to address. Thus a key unanswered hypothesis in this field is that DA oxidation is a major contributor to the death of dopaminergic neurons in PD. The proposed studies address several aspects of this hypothesis including the interaction of known environmental factors in triggering DA oxidation. Specifically, the hypothesis that the DA-releasing potential of the parkinsonism-inducing drug, MPP+, is due to its ability to exchange with DA and/or to reduce intracellular pH gradients will be addressed using newly derived mice expressing enhanced green fluorescent protein from a dopaminergic locus (TH+/eGFP). Primary cultures derived from these animals as well purified synaptosomal and vesicular preparations from dopaminergic terminal fields will be used in combination with fluorescent and radioactive probes to determine the temporal aspects of DA release, intracellular membrane changes, ROS formation, ATP loss, etc in response to toxin treatment. In addition, the hypothesis that DA oxidation contributes to the death of dopaminergic cells will be directly tested in vivo using animals genetically engineered to have different levels of DA production. Behavioral, oxidative and immunocytochemical criteria will be used to establish the role of DA in both the acute and chronic MPTP model of PD. To test whether DA depletion prevents ROS, new methodologies to detect in situ ROS will be used with a battery of antibodies directed against nitrotyrosine, nitrated alpha-synuclein, etc. to temporally evaluate ROS formation following acute or chronic MPTP administration in DA deficient and wild type animals. Taken together, the proposed studies will determine whether DA oxidation plays a central role in the death of DA synthesizing cells and provide insights impossible to obtain from standard animal models. Knowledge of the source and cascade of events surrounding DA-induced free radical formation will help answer risk-benefit controversies surrounding the use of dopamine replacement therapies as well as facilitate the development of new drugs and/or treatment strategies in the pathogenesis of PD. -

Principal Investigator: PALLANCK, LEO J
Grant Number: 5R01NS041780-04
Title: Parkin mediated Neural Dysfunction in Drosophila

Abstract: Parkinson's disease is a prevalent neurodegenerative disorder characterized by tremors, rigidity, and bradykinesia. These symptoms are thought to arise from the degeneration of dopaminergic neurons in the substantia nigra pars compacta. Recently, mutations of the parkin gene, which encodes a ubiquitin-protein ligase, were found to underlie a familial form of Parkinson's disease known as autosomal recessive juvenile Parkinson's disease (AR-JP). While this advance provides clues to the mechanism responsible for pathology in AR-JP, the cellular targets of the parkin ubiquitin-protein ligase activity and the specific biochemical pathways affected by parkin mutations remain largely unknown. To address these issues, the objectives of this proposal are to create a Drosophila melanogaster model of AR-JP through mutational analysis of a Drosophila parkin ortholog, and to use this fly AR-JP model to investigate the molecular mechanisms of neuronal dysfunction underlying parkin deficiency. Two main hypotheses will be explored in this proposal: (a) parkin sequesters alpha-synuclein protein into Lewy bodies and this function represents a cellular mechanism of alpha-synuclein detoxification; (b) neurodegeneration triggered by parkin mutations results from accumulation of parkin substrate(s). To accomplish the objectives of this proposal, the following specific aims will be pursued: (1) Generate and characterize Drosophila parkin (D-parkin) mutants; (2) Determine whether altered D-parkin expression affects the time course and extent of alpha-synuclein induced neurodegeneration and Lewy body formation; (3) Identify modifiers of a D-parkin mutant phenotype; (4) Isolate D-parkin-binding components and investigate structure-function relationships in D-parkin. Results from this work should clarify the relationship between parkin dysfunction and neurodegeneration, and possibly reveal strategies for treatment of Parkinson's disease. -

Principal Investigator: PALLANCK, LEO J
Grant Number: 1R21NS048362-01
Title: Mutational Analyses of Drosophila DJ-1 Homologs

Abstract: Parkinson's disease is a prevalent neurodegenerative disorder characterized by tremors, rigidity, and bradykinesia. These symptoms arise from the degeneration of dopaminergic neurons in the substantia nigra. The cellular and molecular mechanisms responsible for neurodegeneration in Parkinson's disease remain poorly understood, although genetic and environmental factors both appear to play contributing roles. Recently, loss-of-function mutations in DJ-1, a gene of unknown function, were found to be responsible for an autosomal recessive form of Parkinson's disease. To explore the normal biological function of DJ-1, and the mechanism by which loss of DJ-1 function results in neurodegeneration, we propose to subject a pair of highly conserved Drosophila DJ-1 homologs (designated DJ-1a and DJ-1b) to mutational analysis. DJ-1a and DJ-1b function will be perturbed using P element mutagenesis, gene-targeting and double stranded RNA interference methods. The phenotypes resulting from perturbation of these genes will be fully characterized, including an analysis of dopaminergic neuron integrity. Additionally, we will characterize the global gene expression changes resulting from loss of DJ-1a and DJ-1b function and initiate screens for genetic modifiers of the DJ-1a and DJ-1b phenotypes to elucidate the biochemical pathways in which these genes function. This work should clarify the normal cellular role of DJ-1 and provide a foundation for further hypothesis-driven investigation of DJ-1 function. -

Principal Investigator: PAPA, STELLA M
Grant Number: 1R01NS045962-01A1
Title: Regulation of Motor Function in Parkinson's Disease

Abstract: Motor disturbances of Parkinson's disease are caused by a series of functional alterations in the basal ganglia that derive from dopamine denervation. The mechanisms underlying those functional alterations are not completely understood yet. Moreover, long-term levodopa therapy is usually associated with disabling motor complications, such as motor fluctuations and dyskinesias, whose pathophysiology also remains obscure. The long-term objective of this project is to elucidate the pathophysiologic mechanisms of abnormal motor behaviors in Parkinson's disease in view of developing new and specific therapeutic tools. Thus, this study is aimed: -firstly, to localize functional alterations in specific basal ganglia circuits; -secondly, to determine the glutamate regulation associated to an altered neuronal function; -finally, and based on the foregoing data, to explore new therapeutic approaches by interacting with the glutamatergic neurotransmission in a region-specific manner. Specifically this project comprises three aims: 1. To study the neuronal activity of individual basal ganglia regions by single cell recording in normal and various groups of parkinsonian monkeys (MPTP-treated primates) that exhibit different motor behaviors depending on treatment conditions (i.e.: parkinsonian state, its normalization, and drug-induced dyskinesias). 2. To study the glutamate receptor sensitivity in basal ganglia regions in relation to different motor conditions by comparing the binding of receptors across animal groups. 3. To study the glutamatergic blockade in restricted basal ganglia regions by determining its effects on neuronal activity and motor behavior. The research design includes techniques ranging from single- and multiple single- unit recording of neuronal activity, autoradiographic binding of receptors, to intracerebral administration of drugs in parkinsonian monkeys whose motor abnormalities closely resemble the human disease. This project proposes a novel approach to a comprehensive study of the abnormal motor function in Parkinson's disease. Thus, it will largely contribute to the rationale for new treatments that selectively target particular motor conditions. -

Principal Investigator: PARNG, CHUENLEI
Grant Number: 1R43NS048607-01
Title: In Vivo Screen for Neuroprotective Agents

Abstract: Aberrant apoptosis is implicated in several neurodegenerative disorders including, stroke, brain trauma, spinal cord injury, Parkinson's disease, amyotrophic lateral sclerosis (ALS), Alzheimer's and Huntington's disease. These neurodegenerative diseases are associated with high morbidity and mortality, and treatment options are limited. Agents that modulate apoptosis are a major focus of drug development efforts by biopharmaceutical companies. Assessment of drug effects in a convenient vertebrate model, prior to proceeding to evaluation in complex systems, such as mouse, can potentially streamline drug development and dramatically reduce costs. Zebrafish mutants exhibiting aberrant apoptosis in the central nervous system are an excellent animal model for studying neurodegeneration. Using a zebrafish neurodegenerative mutant line and a vital dye apoptosis assay, this Small Business Innovation Research project proposes to characterize embryogenesis and apoptotic patterning in zebrafish embryos, and to develop a rapid and effective in vivo screen for neuroprotective therapeutics.-

Principal Investigator: POIZNER, HOWARD
Grant Number: 2R01NS036449-05A1
Title: Motor Control Deficits in Parkinson's Disease

Abstract: Our findings in the current grant period have led us to hypothesize that a major difficulty for patients with Parkinson disease (PD) is in assembling and using new sensorimotor mappings or coordinations. These process play a major role both in ongoing motor performance and in the acquisition of new skills, in addition, our preliminary data are consistent with a general observation that these processes may be relatively resistant to current therapeutic modalities. Furthering our understanding of this deficit, examining its impact on motor learning, and investigating the ability of dopaminergic therapy to reverse this deficit are the guiding aims of this proposal. The present proposal presents three experiments that are designed to confirm and extend our hypothesis and to investigate the degree to which dopaminergic therapy is able to remediate these deficits. The first two experiments (Specific Aims 1 and 2) introduce the requirement that subjects learn to move within a virtual environment as a prerequisite to establishing the new sensorimotor coordinations necessary for accurate target acquisition. We require subjects to master distortions which create discrepancies between the apparent (virtual) and real (proprioceptively signaled) location of their arms and to generalize the resulting learning to untrained regions of this environment. By dissociating movements from their normal sensory correspondences, we will challenge subjects' abilities to reconfigure their sensorimotor coordinations. The third experiment (Specific Aim 3) challenges patients by requiring them to integrate different motor acts in order to acquire visually-presented, real targets by compensating for a mechanical perturbation of the trunk during a trunk-assisted reach. We have integrated and coupled our previously developed system for analysis and display of three dimensional movements with our newly developed virtual reality environment. We will examine not only subjects' accuracy, but also the path, timing, and structure of their movements under different conditions and types of imposed distortions, in order to measure both performance and learning when PD patients are OFF versus ON dopaminergic therapy. By contrasting the performance and capacities of PD patients on and off dopaminergic therapy to that of comparable normals, we can both obtain clues as to how to overcome PD dysfunction and gain an insight into the key role of the basal ganglia in movement.-

Principal Investigator: REDMOND, D EUGENE
Grant Number: 1U01NS046028-01A1
Title: GDNF Delivery to MPTP Monkeys by EIAV lentivirus and AAV

Abstract: An effective gene therapy for Parkinson's disease is the goal of this proposal, which will test the effectiveness and safety of human glial cell line derived neurotrophic factor (GDNF) delivered by two improved vector systems derived from equine infectious anemia virus (EIAV) or from adenoassociated virus (AAV). Both vectors deliver the cellular marker gene, nuclear localized lacZ (lacZnl) or GDNF efficiently and stably into nigrostriatal target regions, can be regulated using a tetracycline promoter system, and offer additional safety that the respective wild-type viruses do not cause any disease in humans. The recombinant vectors will be tested in the parkinsonian model produced by the neurotoxin MPTP in monkeys. GDNF has shown promise for preventing or reversing morphological, biochemical and functional deficits in other models of Parkinson's disease in rodents and primates, using rAAV, and rHIV. But these studies also showed important problems to be solved to ensure that a GDNF gene therapy will be safe and effective in patients. Concerns about inflammatory, cytotoxic, inadequate or excessive gene expression, persistence, viral recombination or replication have led to the development of improved and safer vectors with regulatable promoters, which will be tested in this proposed project. Initial studies will address transgene expression (lacZnl or GDNF) in normal African green monkeys, determining effective titers, transduction efficiency, cellular tropism, distribution, level, and stability of transgene expression, neuropathology and host cellular responses after delivery by rEIAV or rAAV. Each of the two vectors will then be used to deliver GDNF to the nigrostriatal system of MPTP parkinsonian monkeys to test hypotheses that GDNF expression will improve function in both moderate and severely parkinsonian monkeys for periods up to 24 months. The most effective procedures will be optimized by comparing injection sites, a regulatable promoter to inactivate gene expression, and safety of all procedures including high injection titers. Measures of efficacy will include behavioral parameters, molecular assays of transgene expression using ELISA for protein, RT-PCR for mRNA and PCR for vector DNA, biochemical assays of DA and its metabolites, neuroanatomical and morphometric analyses, neuropathology, clinical chemistry, SPECT imaging, and autoradiography. These studies aim to provide the necessary data to initiate successful clinical trials in Parkinson's patients at the earliest possible time. -

Principal Investigator: REDMOND, D EUGENE
Grant Number: 5R01NS040822-03
Title: Human Neural Stem Cells in Primate Parkinson's Model

Abstract: This project will study the hypothesis that human neural stem cells (hNSCs) implanted into monkeys can normalize parkinsonism resulting from the neurotoxin 1-methyl-4-phenyl-1,2,3,6 tetrahydropyridine (MPTP). These primordial, uncommitted, pluripotent cells can be propagated in large numbers and then safely differentiated into most cell types of the nervous system, including dopamine-producing neurons. NSCs migrate to populate developing or degenerating brain regions, perhaps allowing a more functionally correct and effective reconstruction. Pilot studies now show engraftment of hNSCs in the brain of fetal, neonatal, infant, and adult monkeys, for at least a month. Dopamine depleted adult monkeys showed graft-derived tyrosine hydroxylase positive cells and appropriate migration from the site of injection to dopamine-depleted areas. This project will test hypotheses in monkeys: (1) that hNSCs will survive, differentiate, and integrate in the brain of normal adult monkeys without immunological rejection or harmful overgrowth; (2) that hNSCs will eliminate parkinsonism after MPTP treatment, and that the presence of dopamine injury will influence their distribution and fate. NSCs will be identified and quantitated using genetic markers, immunohistochemistry, and multi-synaptic tract tracing. The following will be characterized and compared in normal monkeys and monkeys after MPTP: hNSC survival, migration, cell division, differentiation, connectivity, immunogenicity, stability of expression of a transgene (LacZ), apoptosis, and effect of host environment on all of these. In the dopamine-depleted parkinsonian monkey, dopamine and its metabolite concentrations, autoradiography of dopamine transporters, behavioral reversal of parkinsonism, dose effects, and synaptic connections will be studied over time courses of 7 days, 1, 3, 6, and 12 months. Comparisons will also be made with effects of primary fetal ventral mesencephalic tissue transplants in parkinsonian monkeys from prior and parallel studies. These studies will advance our understanding of the neurobiology and safety of human neural stem cells in a well established clinically relevant primate model of Parkinson's disease, and, if successful, support safe clinical studies in patients with Parkinson's disease in the future. The results will also advance understanding of useful methods for studying and treating a broad range of neurodegenerative, genetic, and traumatic conditions of the nervous system. -

Principal Investigator: REUBINOFF, BENJAMIN
Grant Number: 5R01NS046559-02
Title: Functional dopamine neurons from ES cells

Abstract: Parkinson's disease is a common neurodegenerative disorder that results from degeneration of dopamine (DA) neurons in the nigro-striatal system. Transplantation of fetal DA neurons can relieve Parkinsonism in some patients; however, limited tissue supply is a major obstacle for widespread use of fetal cells. Human embryonic stem (hES) cells could provide the platform for creating an unlimited supply of human DA neurons for cell therapy of Parkinson's disease. The goal of this study is to develop DA neurons from hES cells (NIH registration code ES01-06) and to demonstrate their function and therapeutic potential in animal models of Parkinson's disease. We have recently developed highly-enriched (>95 percent) cultures of expandable, developmentally competent neural progenitors (NPs) from hES cells. The NPs differentiate spontaneously into neurons expressing tyrosine hydroxylase (TH), however, at a low frequency. Our preliminary data suggest that defined signals can significantly promote the differentiation of hES cell-derived NPs towards TH+ neurons. In this study we will further develop the protocols to direct the differentiation of hES cells into TH+ neurons by the following approaches: (A) Administration of growth factors and cytokines that are known to induce a midbrain fate. (B) Forced expression of key transcription factors in the development of DA neurons. (C) Co-culture with stromal cells that have DA fate-inducing activity. Potential synergism between the strategies will be determined. We will evaluate whether hES cell-derived TH+ neurons have electrophysiological and functional properties expected from midbrain DA neurons and whether they can lead to recovery in the rat model of Parkinson's disease. Our preliminary results suggest that transplantation of hES cell-derived NPs to the DA-depleted striatum of rats results in behavioral recovery of DA-mediated motor asymmetry. Lastly, we will evaluate the potential of hES cell transplantation to correct behavioral deficits and the abnormal electrical activity of basal ganglia neurons in the MPTP primate model, which most reliably mimics the human disorder. This study will pave the way for further developments that may eventually allow the use of human ES cells as an unlimited source of midbrain neurons for transplantation in Parkinson's disease.-

Principal Investigator: SALAMONE, JOHN
Grant Number: 1R01NS047261-01
Title: Dopamine D2 and Adenosine A2A roles:Tremulous Movements

Abstract: Symptoms of parkinsonism, such as akinesia, bradykinesia, and tremor, can be caused by degeneration of dopamine (DA) neurons, or by administration of DA antagonist drugs. Parkinsonism is characterized by a cascade of neurochemical events that reflect interactions between several neurotransmitters in the circuitry of the basal ganglia, including DA, acetylcholine, serotonin, GABA and adenosine. Within the last few years, increasing evidence has accumulated indicating that central adenosine neurons play an important role in modulating the functional circuitry of the basal ganglia. Several subtypes of adenosine receptors are involved in motor function, and anatomical studies have demonstrated that the adensonine A2A receptor subtype has a relatively high degree of expression within the striatum. Although several types of striatal cells contain some adensonine A2A receptors, these receptors are present in very high densities on striatopallidal neurons, which also tend to co-express DA D2 receptors and enkephalin. It has been suggested that antagonists of adenosine A2A receptors could have some potential utility as antiparkinsonian drugs. In a recent study from our laboratory, it was demonstrated that IP injections of the adenosine A2A antagonist, KF17837, also suppressed haloperidol-induced tremulous jaw movements, and reversed the locomotor suppression induced by this D2 antagonist. This profile of activity is consistent with the hypothesis that antagonism of adenosine A2A receptors can result in antiparkinsonian effects in animal models. The proposed experiments are designed to investigate the role of the striatopallidal GABAergic pathway as a possible mediator of the putative antiparkinsonian effects of adenosine A2A antagonists. These proposed studies will focus on the tremulous jaw movement model, which is related to parkinsonian tremor. It is hypothesized that adenosine A2A antagonists are acting on striatopallidal GABAergic neurons that also express DA D2 receptors. In view of research showing that haloperidol increases extracellular GABA in globus pallidus, and that haloperidol-induced tremulous jaw movements are reduced by pallidal injections of bicuculline, it is hypothesized that doses of adenosine A2A antagonists that reduce jaw movement activity also will reduce haloperidol-induced increases in GABA release in globus pallidus. In addition, it is hypothesized that adenosine agonists and antagonists will interact to regulate the behavioral and neurochemical effects of haloperidol. These hypotheses will be investigated using studies that involve both systemic and intrastriatal injections of drugs that act upon A2A receptors, and the proposed work will involve a

Principal Investigator: SANDLER, YAKOV
Grant Number: 5F31NS041852-04
Title: A Model of Dementia with Lewy Bodies

Abstract: The ultimate goal of this proposal is to utilize a Drosophila model of Dementia with Lewy Bodies (DLB) to gain a better understanding of the molecular and genetic mechanisms that lead to the disease state. DLB is a progressive disorder that is the second most common dementia after Alzheimer's disease, accounting for 17-36% of all dementia cases. Clinically, it is characterized by mental state abnormalities such as cognitive impairment, psychosis and recurrent hallucinations. The pathologic hallmarks of the disease are cytoplasmic inclusions, called Lewy bodies (LB), that are widely distributed throughout paralimbic and neocortical regions and contain alpha-Synuclein. Based on familial inheritance studies, mutations of the alpha-Synuclein gene have been associated with LB formation. Misexpression of alpha-Synuclein in both Drosophila and mice results in neuronal loss and inclusion formation. alpha-Synuclein knockout mice have abnormalities of dopamine release. However, neither the exact function of alpha-Synuclein nor its role in the pathogenesis of DLB have yet been elucidated. We plan to investigate the role of alpha-Synuclein in the pathophysiology of DLB using Drosophila eye as an animal model system. This genetic system is powerful and has been successfully used to study other CNS, disorders such as Huntington's Disease and Spinocerebellar Ataxia. In order to gain a better understanding of mechanisms of pathogenesis of DLB, we propose three Specific Aims: (1) To characterize alpha-Synuclein-induced pathogenesis in the Drosophila eye; (2) To study interactions between alpha-Synuclein and chaperones; (3) To screen for genes that affect alpha-Synuclein-induced pathogenesis. The data we obtain from these experiments should give us a better understanding of the molecular mechanisms that lead to mental dysfunction in DLB. Furthermore, identification of genes involved will lead to new, or effective means of preventing, diagnosing and treatin DLB. -

Principal Investigator: SARANG, SATINDER S
Grant Number: 1R43NS050920-01
Title: PESTICIDE-SYNUCLEIN INTERACTIONS AS RISK FACTORS FOR PD

Abstract: Parkinson's disease (PD) and other age-associated neurological disorders represent one of the largest unmet medical needs in developed countries. However, the discovery of improved diagnostics and therapeutics for these disorders is hampered by incomplete understanding of underlying disease mechanisms and risk factors. Oxidative stress, mitochondrial dysfunction, and protein aggregation have been implicated as major mechanisms causing dopaminergic neuronal loss in PD. Epidemiological studies have revealed an association between pesticide exposure and PD, and pesticides that cause oxidative stress and mitochondrial dysfunction, such as rotenone and paraquat, are used in cellular and animal models of PD. Furthermore, interactions between pesticides and the PD-linked gene alpha-synuclein have been postulated. Although almost 1000 pesticide active ingredients are currently marketed, these compounds have not been systematically screened for neurotoxicity in cellular or animal models of PD. The identification of pesticides that interact with alpha-synuclein to cause neurodegeneration may lead to the discovery of novel candidate risk factors and more representative disease models for PD. For this proposal, investigators at Cambria Biosciences will exploit a published moderate-to-high throughput neuronal cell-based model of PD, with the goal of identifying individual pesticides and synergistic pesticide combinations potentially involved in the pathogenesis of PD. Our established cellbased model of PD will be used to screen -approximately 350 registered pesticides to identify neurotoxic pesticides. Our specific aims include: (1a) identifying neurally-active pesticides that induce cell injury to two PD-like cell lines that stably express wild type (WT) human alpha-synuclein and mutant A53T alpha-synuclein; (1b) identifying any synergistic effects of neurotoxic pesticides in inducing cell damage in these a-synuclein-expressing neuronal cells; and (2) characterizing the activity of these neurotoxic pesticides and pesticide combinations using primary mature mesencephalic DA neurons. The identified neurotoxic pesticides will be employed in follow-on Phase II studies for the development of improved in vitro and in vivo PD models, which will ultimately be used to screen for neuroprotective compounds as part of a comprehensive drug discovery program. -

Principal Investigator: SCHLOSSMACHER,
Grant Number: 2K08NS002127-04
Title: The Roles of a-Synuclein and Parkin in Parkinson Disease

Abstract: The pathogenesis of Parkinson disease (PD) is unknown but dopamine-induced oxidative stress, proteasomal abnormalities and mitochondrial dysfunction are associated with its neurodegeneration. Rare heritable forms of PD are linked to an increasing number of gene loci. At the PARK1 locus, SNCA encodes a neuronal protein, alpha-synuclein (alpha-S), that is involved in the transition of synaptic vesicles from the reserve-resting pool to the readily releasable pool in vivo and in vitro. It is linked to sporadic PD by the formation of fibrillar inclusions that contain phosphorylated alpha-S, and to autosomal dominant PD by a likely gain-of-function effect of two infrequent point mutations. The PARK2 gene encodes parkin, an E3 ubiquitin ligase. It is mutated in < 50% of all autosomal recessive PD cases by a probable loss-of-function phenomenon. In normal human brain (but not rat brain), a pool of alpha-S undergoes O-linked glycosylation, thereby generating alpha-Sp22. This glycoprotein is a substrate for parkin's E3 ligase function in vitro and accumulates in PARK2-mutant PD brain. The central hypotheses of this application state that 1) a shared pathogenetic pathway is encoded by PD-linked genes, 2) characterization of the alpha-S glycosylation in primate brain will provide insights into the pathogenesis of PD, 3) the normal function of the Parkin E3 complex is essential for the sustained survival of catecholaminergic neurons in adult human brain, and 4) the identification of the in vivo subunits of the assembled parkin E3 complex will validate reported binding partners and reveal potentially neurotoxic substrates. To this end, I have identified two Specific Aims: Aim 1: To characterize the glycosylation of alpha-S in human control brain as well as PARK1-linked PD brain and to model its biosynthesis in a cell model, and Aim 2: To biochemically purify the subunits of the Parkin E3 ligase complex from human brain, and verify them in vitro.-

Principal Investigator: SCHOR, NINA F
Grant Number: 5R01NS041297-03
Title: Antioxidant Strategies for Parkinson's Disease

Abstract: Reactive oxygen species (ROS) have been implicated in the pathogenesis of Parkinson's disease. This suggests that antioxidant strategies may be useful in the treatment and/or prevention of this neurodegenerative disorder. We have developed and implemented two models for the central movement disorder and autonomic peripheral neuropathy, respectively, associated with Parkinson's disease. We propose to use these models to design and test antioxidant strategies we have previously developed for adjunctive use with ROS-generating chemotherapeutic agents. We will further use our studies of the biochemical effects of antioxidant treatment to develop a screening test for new antioxidant agents for use in Parkinson's disease and other ROS-related disorders. Specifically, we propose to test the hypothesis that recycling antioxidants increase expression of p21 wafl/cip1,enhance binding of HIF-1 and CREB to DNA, activate NF-kappaB, prevent ROS-induced morphological apoptosis, and decrease ROS-induced membrane phospholipid and protein nitration in culture models of Parkinson's disease. We will further test recycling antioxidants for their distribution to the CNS and peripheral compartments, and use this information to test CNS-penetrating and non-CNS-penetrating agents for efficacy in the central and autonomic nervous system models, respectively, of Parkinson's disease. Finally, we will test the hypothesis that the magnitude of induced in vitro biochemical change for each drug correlates with the degree of protection from the effects of ROS in the CNS or autonomic model. This latter study will pave the way for development of an in vitro screening test for new antioxidant strategies proposed for use in Parkinson's disease. This application specifically addresses the NINDS agenda for research in Parkinson's disease in its development of in vitro screening tests for putative therapeutic agents in general and antioxidants in particular for this disease, its development of animal models for the clinical aspects of Parkinson's disease, and its potential for further elucidation of the mechanisms of ROS-induced apoptosis in the nervous system.-

Principal Investigator: SHEN, JIE
Grant Number: 5R01NS041779-04
Title: Studies of Parkin KO Cells and Mice as PD Models

Abstract: Parkinson's disease (PD) is an age-related neurodegenerative disorder affecting approximately 5% of people over age 65. PD is characterized pathologically by the selective degeneration of dopaminergic neurons in the substantia nigra and the formation of intraneuronal inclusions known as Lewy bodies. Recessively inherited mutations in the Parkin gene are the most common cause of inherited and early onset PD. A variety of large Parkin deletion and truncation mutations as well as missense mutations have been linked to PD in many families, strongly indicating that recessively inherited parkinsonism is caused by loss of Parkin function. The central hypothesis underlying our research is that loss-of-function mutations in the Parkin gene alter the normal physiology of dopaminergic neurons in the substantia nigra, ultimately leading to the parkinsonian phenotype. A loss-of-function pathogenic mechanism can be studied in cells and animals from which the Parkin gene has been deleted. Knockout mice are commonly used to investigate the normal function of genes. Knockout mice can also be used to study diseases caused by gene deletions in humans. Parkin knockout mice can be used to study the abnormal nigral degeneration caused by loss of Parkin function in humans. To investigate the role of Parkin in the survival of dopaminergic neurons, we propose to generate mice with targeted germ-line disruption of the Parkin locus. The Parkin knockout mice will then be analyzed for biochemical and neuropathological abnormalities associated with PD, such as degeneration of dopaminergic neurons, reductions in striatal dopamine levels, and motor behavioral deficits. In parallel, we will generate and analyze Parkin knockout cells in vitro. This will provide a powerful cellular system with which to characterize the function of Parkin and to examine the consequences of its absence, such as increased sensitivity to oxidative stress and apoptotic stimuli. Both the animal and the cellular systems could provide valuable means for identifying and testing molecules and genes with therapeutic potential. -

Principal Investigator: SHEN, JIE
Grant Number: 5R01NS041783-04
Title: PS-1 in APP Processing and Synaptic Function

Abstract: The goal of the proposed project is to define the function of presenilin-1 (PS I) in the adult brain, including its role in the processing of the amyloid precursor protein (APP), Notch signalling, and synaptic function. Our previous studies of PS1-/-mice revealed a critical role for PS1 in neurogenesis, neuronal migration and Notch signalling during neural development. The perinatal lethality of PS1-/- mice precludes the analysis of PS1 function in the adult brain. We therefore developed a viable conditional PS1 knockout (cKO) mouse using the Cre/loxP recombination system, in which PS1 function is selectively inactivated in neurons of the postnatal forebrain. Here we propose to use the cKO mouse to investigate the role of PS1 in APP processing, generation of b-amyloid peptides and amyloid plaque formation in the adult cerebral cortex. We will also investigate whether disruption of PS 1 function in the adult brain leads to a reduction in Notch signalling as it does in the embryonic brain. Lastly, we will address whether PS1 is required for neuronal survival, synaptic transmission and plasticity, as well as learning and memory. The significance of the proposed study is that it will elucidate the normal physiological role of PS1 in the adult brain and test the feasibility and suitability of targeting PS1 for anti-amyloidogenic therapy in Alzheimer's disease. -

Principal Investigator: SMEYNE, RICHARD J
Grant Number: 2R01NS039006-04A2
Title: Genetics of MPTP-Induced Parkinsonism

Abstract: Parkinson's disease (PD) is a debilitating neurological disorder that strikes 20 per 100,000 persons greater than 50 years of age. It is estimated that 1 million US citizens have PD, with adults over 60 having a 1 in 20 chance of getting PD. At an average per capita cost of $6000.00 year/patient, the total cost of the disease approximates $6 billion dollars, of which 85% is borne to private and government insurance agencies. Since the population of the world is getting progressively older, the number of people suffering from this disease should substantially increase within the next several decades. The cause of >90% of all PD cases is unknown. Current hypotheses on the etiology of idiopathic PD (IPD) state that there is an interaction of some as yet unknown environmental agent with a genetic predisposition to its effects. The discovery of 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided a useful model of Parkinsonism that appears to recapitulate the pathology of the disease seen in man. Exposure to this prototypical "environmental toxin" causes a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). In mice, the effects of MPTP are strain dependent. We have used a QTL analysis to demonstrate that the gene underlying strain differences is located on chromosome 1. Within this chromosomal region, one gene: glutathione-S-transferase pi2 functions within the detoxification pathway for exogenous agents. In this application, we propose to study the structure and function of this gene and its related family members. Four specific aims are proposed: 1) Determine if there are any differences in the sequence and expression of GSTp2 and related family members in MPTP-resistant and sensitive strains of mice. 2) Examine the effects of blockade or transfer of GSTpi on cell death following administration of MPTP in vitro and in vivo; 3) Develop the rotenone model of experimental Parkinsonism in mice and determine if GSTp2 is altered in response to rotenone; 4) Determine if there are structural or expression differences in GSTpi levels in humans with Parkinson's disease. The results of this study should lead to a better understanding of the pathogenesis of experimental and possible human Parkinson's disease. This identification of GSTp2 as a candidate gene could also lead to the identification of diagnostic measures and point to potential therapies for early intervention in this devastating illness. -

Principal Investigator: SMEYNE, RICHARD J
Grant Number: 1R21NS045906-01A2
Title: Role of Environment in Neuroprotection

Abstract: PD is a debilitating neurological disorder that strikes 20 per 100,000 persons greater than 50 years of age. The cause of >90% of all PD cases are unknown. However, the discovery of the meperidine by-product 1-methyl-4-phenyl-1,2,3,6-tetrahydropyridine (MPTP) has provided a useful model of Parkinsonism that appears to recapitulate the pathology of the disease seen in man. Exposure to this prototypical "environmental toxin" causes a selective loss of dopaminergic neurons in the substantia nigra pars compacta (SNpc). MPTP is a lipophillic molecule that rapidly enters the brain and is metabolized to MPP+ through a series of intermediates to MPP+ by the enzyme MAO-B. MPP + is a substrate for dopamine uptake mechanisms and it accumulates intraneuronally and interferes with complex I of the electron transport chain. We have recently shown that the glial cell is the critical cell for conferring protection or susceptibility to this toxin. Since PD is progressive, both in terms of cell loss and symptomotology, it would be of tremendous clinical value if there were cell biological, pharmacological or non-pharmacological methods that could attenuate cell loss; with or without interruption of the disease triggers. Alternatively, at the least, it would be important to slow the progression of cell loss once symptoms arose. There is a significant literature, dating back to the late 1700's that altering an animals' environment can lead to neurological changes. These changes are manifested as increased brain size, increased learning, and recently it has been shown that environment can increase neurogenesis. Recently, we have preliminary data to suggest that mice raised in an "Enriched Environment" (EE) are protected from MPTP toxicity. In this application, we will study and further establish the EE model. In addition, we will examine if the components (exercise, alterations in environmental complexity and/or social interactions) of the EE can confer neuroprotection. In addition, we will examine the role of the neurotrophin BDNF in EE-dependent neuroprotection. The work proposed and subsequent results generated in the application will be used as pilot data. We believe that the EE model may provide a new approach to prevention of PD symptomatology as well as other neurodegenerative disorders. -

Principal Investigator: SOGHOMONIAN, JEAN-
Grant Number: 5R01NS040783-03
Title: Behavioral Sensitization and Parkinson's Disease

Abstract: The systemic administration of agonists of dopamine receptors remains one of the most effective therapeutic interventions used for the symptomatic treatment of Parkinson's disease. However, the chronic administration of these agonists over several months-years can induce the gradual development of debilitating abnormal involuntary movements such as dyskinesia. Current models of the basal ganglia favor the hypothesis that the chronic administration of dopaminergic agents involves an increased/abnormal GABA signaling in the substantia nigra, pars reticulata (SNr), and in the internal pallidum. We propose to examine this hypothesis in a rodent experimental model of Parkinson's disease. The specific aims are: 1-To test the hypothesis that the chronic administration of agonists of dopamine receptors to 6-OHDA-lesioned rats alters the expression of molecules involved in the regulation of GABA levels in neurons that provide an input to the SNr/internal pallidum; 2-To test the hypothesis that increases in basal extracellular GABA levels in the SNr/internal pallidum are involved in the effects of long-term administration of agonists. 3-To test the hypothesis that plasticity of GABA receptors in the SNr plays a role in the effects of chronic administration of dopaminergic agents. These studies will involve quantitative in situ hybridization histochemistry to measure changes in mRNA levels, microdialysis to measure changes in GABA levels and intranigral administration of pharmacological agents acting on GABA levels or GABA receptors to alter agonist-induced circling in rats unilaterally lesioned with 6-OHDA. -

Principal Investigator: Sonsalla, Patricia K
Grant Number: 5R01NS041545-04
Title: Dopamine Homeostasis, Vesicles & Neurodegeneration

Abstract: Parkinson's disease is a debilitating motor impairment disorder due to loss of nigral dopamine neurons. Mitochondrial defects in PD patients implicate energy impairment and metabolic stress as potential factors in its etiology. Moreover, DA oxidation products may add to the oxidative burden within DA neurons which, coupled with a persistent metabolic stress, may lead to neurodegeneration. Epidemiological studies link PD with environmental exposure to substances such as pesticides. - Many pesticides are mitochondrial inhibitors. A potential form of protection against mitochondrial toxins (i.e., MPP+) may be their sequestration into synaptic vesicles of DA neurons. The goal of this project is to gain an understanding of the role of vesicles, the vesicular monoamine transporter (VMAT2) and DA in modulating neurodegeneration produced by mitochondrial toxins. One hypothesis is that the actions of mitochondrial toxins can be modulated in contrasting ways depending on whether the toxins are sequestered into vesicles. If sequestered, toxin exposure could be abrogated. In contrast, disruption of vesicular function toxin could lead to disturbed DA homeostasis and enhanced toxicity since it would remove the toxin from interaction with mitochondria. In Aim 1 several mitochondrial toxins will be examined for their ability to interfere with vesicle function (i.e. to inhibit DA uptake into isolated rat membrane vesicles). In aim 2, rat mesencephalic cultures or rat striata will be exposed to mitochondrial toxins following VMAT2 inhibition to determine if toxicity is modified. To examine the hypothesis that disturbed DA homeostasis contributes to degeneration produced by metabolic stress, two approaches will be used. First, DA will be depleted prior to exposure of culture or rat striata to a mitochondrial inhibitor. Second, vesicle contents (DA) will be released into the cytosol after exposure to the mitochondrial toxin to examine if augmented disruption of DA homeostasis during the metabolic stress enhances toxicity. Additionally, the hypothesis that substances that are not themselves mitochondrial inhibitors, but can disrupt DA storage in vesicles may amplify damage during episodes of metabolic stress will be examined in Aim 3. In aim 4 the hypothesis that early events such as oxidative stress leads to loss of vesicle function, disruption of DA homeostasis and exacerbation of neurodegeneration produced by toxins will be investigated. Isolated vesicles will be tested for their sensitivity to oxidizing and reducing conditions. Results from these studies will provide novel and relevant information as to the contribution of VMAT2 containing vesicles in neuroprotection as well as in neurodegeneration of DA neurons during metabolic

Principal Investigator: STARR, PHILIP A
Grant Number: 2K08NS002201-04A1
Title: Pallidal Physiology in Human and Primate Dystonia

Abstract: Dystonia is a movement disorder defined as a syndrome of sustained muscle contractions, causing twisting and repetitive movements, and abnormal postures. It is often devastating and its pathophysiology is poorly understood. Recently, attempts have been made to understand movement disorders in terms of alterations in a loop circuit involving the cortex, basal ganglia and thalamus. The globus pallidus internus (GPi) occupies a critical position in this circuit since it is the major output structure of the basal ganglia. Another movement disorder, Parkinson's disease (PD), has been found to be associated with excessive and abnormally patterned GPi activity. This finding has led to improved surgical treatments for PD by pallidal inactivation. In contrast to PD, a better understanding of dystonia has been hampered by a lack of data on the physiology of the basal ganglia in this condition, and by the lack of a well-characterized nonhuman primate model of dystonia. Both problems are addressed in this ongoing study. In the initial three years, we recorded and analyzed 283 pallidal units in 14 patients with dystonia, 74 units in a normal Rhesus macaque, and 75 units from four patients with Parkinson's disease. Human patients undergo electrophysiologic mapping as a routine part of pallidal surgery for movement disorders. We showed that, in comparison with normal macaque, dystonia is associated with reduced neuronal activity in the GPi in most but not all cases, increased bursting activity in GPi, and a slight reduction in activity in the external pallidum. These data lend support to a model of dystonia in which both direct and indirect pathways of the basal ganglia are overactive. However, some cases show little abnormality in discharge rate or pattern, motivating a continued search for a "signature" abnormality in dystonia. In addition, we began development of a model of focal arm dystonia in the Rhesus macaque, in which dystonia is generated by repetitive performance of a skilled motor task. In the proposed continuation, spontaneous and movement-related discharge in GPi will be studied in ten additional dystonia patients, with a new emphasis on neuronal responses to sensory feedback and cross correlation of simultaneously recorded cells. In the macaque model of dystonia, the effect on motor performance of lesioning the globus pallidus will be analyzed. The experiments test the following hypotheses: 1) Idiopathic dystonia in humans is associated with abnormal neuronal synchrony and abnormal responses to somatosensory examination in the GPi. 2) In non-human primates, dystonia induced by a repetitive arm movement task can be ameliorated by lesions of the GPi, establishing the relevance of this model to human

Principal Investigator: STEECE-COLLIER, KATHY
Grant Number: 5R01NS045132-02
Title: LEVODOPA DYSKINESIAS: IMPACT OF DOPAMINE NEURONS

Abstract: Recent findings from long-term clinical grafting trials for Parkinson's disease (PD) show that a portion of graft recipients develop aggravated post-graft dyskinesias. These dyskinesias are severe, debilitating and strongly indicate that mechanisms underlying them need to be elucidated. Freed, Fahn and coworkers have hypothesized that grafted-mediated dyskinesias result from graft overgrowth. However, their own PET and post-mortem data, as well as the data from others, do not support this view. We propose an alternative hypothesis that post-graft worsening of dyskinesias result from local "hot spots" of hyperdopaminergic function interacting with the levodopa primed brain. We plan to test this hypothesis by comparing neural grafting strategies that induce either a) widespread or b) local hyperdopaminergic function upon dopa-induced dyskinesias AND the role of dopa priming in a rat model of parkinsonism. We, and others have demonstrated that unilaterally dopamine (DA) depleted rats chronically treated with levodopa exhibit dyskinesias with characteristics remarkably similar to the dyskinesias seen in human PD. Further, this animal model importantly displays basal ganglia mechanisms that allow for DA grafts to either accentuate (Steece-Collier et al, submitted) or ameliorate these dyskinesia indices, similar to that seen in human graft recipients. Prior to continued clinical use, a systematic evaluation of the interaction of neural grafting with levodopa dyskinesias is needed to ensure that this experimental therapy is both safe and effective. This rodent model provides a valuable first step in such a systematic evaluation of levodopa/graft interactions. These studies will provide important guidelines useful in developing primates studies where further hypotheses and verification can be tested. -

Principal Investigator: SUBRAMANIAN,
Grant Number: 7R01NS042402-04
Title: Intranigral Transplantation in Parkinsonian Monkeys

Abstract: Recent investigations indicate that dopaminergic (DArgic) neurons in the substantia nigra (SN) secrete dopamine not only in their axonal terminals within the striatum but also via their dendrites within the SN pars reticulata (SNr) and that loss of dopamine in the SNr may have a role in the development of parkinsonism in primates. As a corollary, restoration of both nigral and striatal dopamine inputs may produce better recovery of function in Parkinson's disease than restoration of dopamine inputs in the striatum alone. Therefore, the PI proposes to examine the effects of combined DArgic fetal ventral mesencephalic (FVM) cell transplantation into the SN and the striatum in 1-methyl-4-phenyl-1, 2, 3, 6-tetrahydropyridine (MPTP)-treated hemiparkinsonian (HP) monkeys and compare the results with FVM transplants in the striatum or SN alone. Animals will be periodically assessed by investigators blinded to the type of transplantation using a behavioral battery of tests (BBT). All animals will be treated with intracarotid MPTP injections to cause a stable HP state and briefly treated with oral levodopa to verify responsiveness to DArgic therapy prior to randomization into 4 equal groups (1-4). Microelectrode recordings of neuronal activity and magnetic resonance imaging (MRI) will be used to guide all transplantation procedures. In specific aim 1 (SA 1), group 1 animals will receive simultaneous FVM transplants into both striatum and the SN, group 2 animals will receive striatal FVM transplants, group 3 animals will receive FVM transplants into the SN and group 4 animals will receive "control" fetal tissue transplants into the SN. Periodic BBT assessments and immunochemical assessment of the transplanted animals compared across groups 1-4 will be used to test the hypothesis that combined striatal and nigral FVM transplants ameliorates parkinsonism to a greater extent than striatal FVM or nigral FVM transplants alone. In SA 2, neuronal recordings will be obtained before and after tissue transplantation from all 4 groups of animals from the SNr and the subthalamic nucleus (STN) and compared. This experiment will examine the hypothesis that striatal FVM transplantation will alter neuronal discharge patterns in both SNr and in the STN, while nigral FVM transplantation will alter neuronal discharge patterns in the SNr only. In SA 3, dopamine levels will be measured in vivo using microdialysis before and after nigral FVM transplantation from the SN and STN in group 3 and group 4 animals. This experiment will test the hypothesis that nigral FVM transplants restore dopamine content in the SN but do not effect dopamine content in the STN. These 3 experiments will objectively evaluate the role of restoring DArgic

Principal Investigator: SURMEIER, DALTON
Grant Number: 5R37NS034696-10
Title: DOPAMINERGIC AND MUSCARINIC SIGNALING IN THE STRIATUM

Abstract: Parkinson's disease (PD) is a disabling neurodegenerative disorder that is expected to affect as many as 1,000,000 Americans this year. Human and animals studies have shown that parkinsonism results from the degeneration of nigrostriatal dopaminergic neurons. Currently, treatment strategies for PD patients are limited. Gaining a better understanding of how striatal function is altered by the disease should broaden the range and efficacy of treatments. In the last funding period we focused on how dopamine and acetylcholine modulate the properties of voltage dependent ion channels in identified normosensitive striatal neurons. These studies have provided fundamental new insights into how these neuromodulators control the excitability of striatal neurons. Now, we are in a position to take the next step toward understanding the pathophysiology of PD - namely, how does the depletion of intrastriatal dopamine alter striatal function? Simply stated, our central goals are 1) to determine how DA depletion alters the regulation of voltage-dependent ion channels in striatal medium spiny neurons and 2) to determine how this adaptation alters their integrative, state-dependent behavior. To this end, we will determine how DA depletion alters Na+ and Ca2+ currents and their modulation by D2 (Specific Aim 1) and D1 receptor activation (Specific Aim 2) in identified striatal neurons. These experiments will rely upon voltage-clamp and single cell reverse transcription-polymerase chain reaction (scRT-PCR) approaches in acutely isolated striatal neurons - techniques with which we have an established track record. The proposed studies will employ newly developed mouse transgenic models in which striatal dopamine levels are profoundly reduced, mimicking the state found in advanced PD. Adaptations in the signal transduction pathways linking receptors to channels will be characterized using a combination of pharmacological, molecular and transgenic strategies. Inferences drawn from this work about adaptations in the mechanisms governing state transitions and repetitive spike activity will be explicitly tested using current- and voltage-clamp techniques in a novel corticostriatal slice preparation where medium spiny neurons exhibit state-dependent behavior resembling that seen in vivo (Specific Aim 3). -

Principal Investigator: Swanwick, CATHERINE
Grant Number: 5F31NS043831-03
Title: BDNF and Synaptic Plasticity in Levodopa Sensitization

Abstract: Currently the most effective treatment for Parkinson's disease (PD) is levodopa. However, for many patients the benefit of levodopa treatment is limited by the development of levodopa-induced dyskinesias over time. The proposed experiments test the principal hypothesis that BDNF induces levodopa sensitization in the 6-OHDA lesioned rat model of PD through modulation of striatal LTP. The experiments address two specific aims: 1) to demonstrate the existence of synaptic plasticity in the denervated striatum after levodopa sensitization and 2) to establish the role of BDNF as a modulator of this plasticity. For Specific Aim 1, synaptic efficacy will be measured in medium spiny neurons of the denervated striatum using evoked field potential recordings. The NMDA receptor antagonist APV will then be applied to test of this synaptic efficacy is NMDA receptor-dependent. For Specific Aim 2, in situ hybridization will be used to examine the expression of BDNF and its receptor TrkB in the striatum and the cerebral cortex of levodopa-sensitized rats. Evoked field potentials will then be measured both when exogenous BDNF is applied to unsensitized denervated striata and when TrkB-IgG fusion protein, a scavenger of endogenous BDFN, is applied to sensitized denervated striata.-

Principal Investigator: TESTA, CLAUDIA M
Grant Number: 5K08NS044267-03
Title: Mitochondrial dysfunction in neurodegenerative disease

Abstract: Like most neurodegenerative disorders, Parkinson disease (PD) has a chronic, slowly progressive course, selective neuronal loss, and a small percentage of familial cases caused by mutations in widely expressed genes. A simplified, reproducible and relevant model system that allows study of progressive neuronal injury would permit us to examine mechanisms of chronic neurodegeneration in PD, and allow us to screen potential neuroprotective agents. Organotypic "slice" culture models offer major advantages in that they are simplified compared to in vivo models, yet unlike dissociated cell cultures they involve the use of mature neurons, remain viable in culture for months, and maintain substantial intact circuitry and neuronal-glial interactions. We propose to characterize and use such a model to specifically examine mechanisms of neuronal injury in PD. Mitochondrial dysfunction has been proposed as a factor underlying dopaminergic cell loss in PD. There is growing evidence of decreased mitochondrial function and increased oxidative stress in human PD. In a new animal model of PD, systemic infusion of the mitochondrial toxin rotenone, an organic pesticide, causes degeneration of the nigrostriatal pathway that is highly selective, even in the presence of global mitochondrial inhibition. In the current proposal we will: 1) Optimize and characterize a rotenone model of PD in chronic organotypic slice cultures. We present data from preliminary studies demonstrating the successful use of slices containing substantia nigra pars compacta dopaminergic neurons for this purpose. 2) Exploit the unique advantages of this system to investigate the mechanisms of action of mitochondrial inhibition. We will examine the role dopamine itself plays in neuronal vulnerability, and look for evidence of oxidative damage and apoptotic cell death. 3) Investigate the interaction of genetic defects with environmental stressors in PD. We will use transgenic mouse models to examine how rotenone interacts with genetic mutations that produce familial PD. We will study how underlying genetic lesions that affect oxidative stress and apoptosis pathways may predispose cells to damage from exogenous toxins. 4) Test potential neuroprotective agents in a model of chronic neurodegeneration that is highly relevant to PD. The research outlined above is part of a customized five-year plan of training and career development for the Principal Investigator. The proposal includes active mentoring by experienced scientists, access to diverse resources, and an environment uniquely suited to help the PI develop as an independent physician scientist.-

Principal Investigator: TKATCH, TATIANA
Grant Number: 1R21NS048524-01
Title: RNAi Targeting of Kv3 Channels in Basal Ganglia Disease

Abstract: Parkinson's disease (PD) is a neurodegenerative disorder characterized by impairment of motor function. It affects about 1 in 1000 adults, rising exponentially after the age of fifty. At present there is no treatment for PD shown to definitively attenuate disease progression. Even temporal correction of symptoms extending the period of physical mobility is considered valuable. We suggest to test a new strategy to relieve motor symptoms of the Parkinson's disease. The abnormal correlated rhythmic activity in the globus pallidus (GP) and subthalamic nucleus (STN) are believed to underlie bradykinesia and tremor of PD patients. A specific set of membrane conductances in GP and STN neurons enable such activity. Recent work by our group has shown that high frequency burst discharge in GP and STN neurons is dependent upon their expression of a combination of voltage-dependent Kv3 K+ channel subunits. These neurons form heteromeric channels containing Kv3.1 and Kv3.4 subunits. These heteromeric channels are very efficient at repolarizing spikes - keeping them very brief - and then deactivating after the spike to allow the next spike to occur quickly. Eliminating the Kv3.4 subunit from these channels diminishes the repolarizing efficiency of the channels, resulting in lower maximal discharge rates. Thus our goal is to test the hypothesis that the suppression of Kv3.4 subunit in GP/STN neurons will dramatically reduce pathological, high frequency burst discharge leading to symptomatic relief in PD models and patients. Kv3.4 is an excellent target for gene therapy approaches since its expression is highly specific for fast spiking neurons and the firing of non-targeted neurons in GP/STN surrounding areas should not be affected. We propose to use lentivirus vector to deliver small interfering RNA (siRNA) designed to trigger the degradation of Kv3.4 mRNA in GP and STN neurons. The proposed specific aims will allow development of the technology that is necessary for testing of our hypothesis in the animal models of PD. -

Principal Investigator: TROYER, MATTHEW D
Grant Number: 5K08NS002251-05
Title: OXIDATIVE STRESS/ALPHA-SYNUCLEIN IN PARKINSON'S DISEASE

Abstract: Parkinson's disease (PD), the second most common neurodegenerative disorder, results from selective loss of midbrain dopamine neurons. Both oxidative stress and intracellular aggregation of proteins, including the protein alpha-synuclein, are implicated in this degeneration. However, the source of oxidative stress, the mechanism of alpha-synuclein deposition, and the relationship between them are unknown. We will examine the role of the neurotransmitter dopamine in generating oxidative stress by manipulating its synthesis, degradation and vesicular transport and measuring production of reactive oxygen species. We will also investigate the effect of dopamine-mediated oxidative stress on alpha-synuclein deposition in model cell culture systems. We will then use this information to determine conditions that promote alpha-synuclein deposition in transgenic mice expressing wild type human alpha-synuclein or a mutant alpha-synuclein that causes an autosomal dominant form of PD. Thus we hope to identify factors that promote oxidative stress in dopamine neurons and to better understand the mechanisms and significance of alpha-synuclein deposition in PD. This work will be conducted under the sponsorship Dr. Robert Edwards in the Departments of Neurology and Physiology at UCSF. Through this work I will learn new techniques in molecular biology, biochemistry and imaging that I will continue to apply to PD. Dr. Edwards and I have developed a training program that includes laboratory research, coursework and didactics that will enable me over the next five years to become an independent investigator concentrating on the neurobiology of PD. In the long term I intend to spend 75% or more of my time dedicated to scientific investigation of PD, and to direct my clinical activities toward patients with PD and other movement disorders. -

Principal Investigator: TURNER, ROBERT S
Grant Number: 5R01NS044551-03
Title: DBS AND MOTOR CORTICAL FUNCTION IN AN MPTP MODEL OF PD

Abstract: Deep brain stimulation (DBS) of either the internal segment of the globus pallidus (GPO or the subthalamic nucleus (STN) is an effective treatment for most if not all symptoms of Parkinson's disease (PD). Several aspects of the reduction of symptoms with DBS provide tantalizing hints that different symptoms may be mediated by distinct pathways and/or physiological processes involving the motor and premotor cortices. The goals of this project are to use a non-human primate model of PD to gain a better understanding of the cortical mechanisms by which DBS produces clinical benefit, as well as to determine if different symptoms have different neuroanatomic/physiologic substrates. Animals will perform tasks that measure symptom-relevant behavioral parameters: movement selection/initiation/sequencing (akinesia), movement kinematics (bradykinesia), and rigidity. Neuronal activity at multiple locations in the four principal motor cortices [in different animals, primary motor (M1), ventral premotor (PMv), dorsal premotor (PMd), or mesial premotor (SMA)] will be monitored using a multielectrode array. Single cell activity will be assessed for changes in resting firing rate, task-related activity, and cell-to-cell interactions (synchronized firing) in response to DBS in GPI or STN before and after animals are rendered parkinsonian by intracarotid infusion of MPTP. The predictions are that: DBS-related changes in resting discharge will not be correlated with specific changes in symptoms. Increased activity and synchrony in SMA will be associated with reduced akinesia. Increases of the same in M1 will accompany reduced bradykinesia. Reductions in rigidity will be linked with a drop in M1 responses to passive movement and increased directional specificity in movement related activity. In addition, DBS may reduce abnormally-increased activity in PMv and PMd These hypotheses will be tested in three specific aims: Specific aim I will study the interacting effects of DBS and the type of motor task being performed. Specific aims 2 and 3 will identify cortical activities that change in concert with the time course (SA 2) and parametric relations (SA 3, DBS location, frequency, and strength) of symptom reduction with DBS. The results of these experiments will improve understanding of both the neuronal basis of different symptoms of PD and the mechanisms of action of DBS. Ultimately, these studies will advance a more complete pathophysiologic model of PD by incorporating the full array of parkinsonian symptoms.-

Principal Investigator: VAN DER WALT, JOELLE
Grant Number: 1L30NS050033-01
Title: Mitochondrial dysfunction in Parkinson's disease

Abstract: Unavailable

Principal Investigator: VAN HORNE, CRAIG G
Grant Number: 1R41NS047959-01
Title: Objective measures of speech post-L-dopa & STN DBS in PD

Abstract: Speech is a complex motor behavior often disrupted by neurologic dysfunction. Parkinson's disease (PD) is one of the most prominent neurological disorders associated with speech disturbances. L-dopa continues to be the cornerstone of medical treatment for the motor symptoms and typically produces some improvement of speech symptoms. As the disease progresses, medical management becomes increasingly difficult and is associated with disabling side effects. Recently, deep brain stimulation (DBS) has been shown to be an effective adjunct therapy for control of the motor symptoms in select patients with advanced disease. The clinical effects of L-dopa and DBS on speech have not been consistent, and some studies report a substantial worsening of speech following these procedures. Many of the difficulties in evaluating speech arise from the subjective nature of evaluation. Even standardized protocols administered by trained professionals demonstrate significant inter-rater variability. We propose to develop a cost effective, portable, stand-alone technology package to analyze multiple components of recorded speech. We will study the applied technology in PD patients and analyze the effects of L-dopa and DBS on objective measures of speech. We will also expand our speech assessment technologies by adding new analytical tools based on sensitive, non-linear dynamic algorithms.-

Principal Investigator: VANCE, JEFFREY M
Grant Number: 5R01NS031153-11
Title: Genomic Screen To Identify Alzheimers Disease Genes

Abstract: To identify genes influencing age at onset (AAO) in two common neurodegenerative diseases, we performed a genomic screen for AAO in families with Alzheimer disease (AD;) and Parkinson disease (PD. (Li et al, AJHG, April, 2002). Heritabilities between 40 percent-60 percent were found in both the AD and PD datasets. For PD, significant evidence for linkage to AAO was found on chromosome 1p (LOD =3.41). In addition, evidence for AAO linkage on chromosomes 6 and 10 was identified independently in both the AD and PD data sets. Subsequent unified analyses of these regions identified a single peak on chromosome 10q between D10S 1239 and D10S 1237, with a maximum LOD score of 2.62. These data suggest that a common gene affects AAO in these two common complex neurodegenerative diseases. We propose to further map and identify the genes contributing to this age-of-onset effect. We will continue to collect new AD and PD families to further map the peaks, and test candidate genes within the region for association to age of onset in these two disorders. Candidates will be prioritized using initially obvious biological candidates, then candidates that lie within the linkage peaks that are identified through Serial Analysis of Gene Expression and Microarray studies in both AD and PD (being performed in our lab in concurrent studies) and finally through fine mapping of the linkage peak for high areas of association using a DNA pooling approach and a new Single base pair- denaturing high performance liquid chromatography methodology. Candidates lying within these high association areas will be investigated further. Once identified, the genes will be investigated in collaboration with known mouse models, at present the Parkin model of Dr. Jian Feng and the APOE models of Dr. Don Schmechel of the DUMC Alzheimer Disease Research Center. Identifying age-of-onset genes may lead to treatment and delay of these late-onset disorders and a better understanding of the pathological processes they share.-

Principal Investigator: VITEK, JERROLD L
Grant Number: 7R01NS037019-06
Title: Deep Brain Stimulation in the Parkinsonian Monkey

Abstract: Over the last decade, the outlook for patients with advanced parkinsonism and other movement disorders has been revolutionized by the introduction of deep brain stimulation (DBS) in the subthalamic nucleus (STN) and internal segment of the globus pallidus (GPi) as a highly effective treatment modality. According to recent estimates over 2000 patients with PD have undergone implantation of DBS electrodes for the treatment of PD and over 15,000 patients per year may be candidates for this procedure. This number will increase, as the use of DBS as treatment of brain disorders becomes more widespread. Despite their widespread use, very little is known about the physiologic effects of DBS. Given the somewhat similar effect of lesions and stimulation in STN, GPI and thalamus on parkinsonian motor signs, it has been speculated that stimulation may act similar to lesioning, by blocking neuronal activity. Several studies have supported this view reporting suppression of neuronal activity in the site of stimulation. Our preliminary results, as well as the results of other groups have suggested that stimulation may, in fact increase output from the stimulated structure, demonstrating that stimulation in the STN increases neuronal activity in the GPi, while GPi stimulation suppresses neuronal activity in the thalamus. Additional support for this hypothesis is derived from microdialysis studies that found increased levels of glutamate in the entopeduncular nucleus (the rodent equivalent of GPi in primates) during STN stimulation. Conceivably, stimulation of basal ganglia activity may improve parkinsonism simply by regularizing pallidal discharge patterns. Both activation and inactivation could, in fact, be invoked during stimulation, because electrical stimulation may inhibit neuronal activity, while activating fibers in the stimulated area. For further optimization of current DBS protocols, and to minimize risks and side-effects of DBS implantation, it is mandatory that a solid understanding of the mechanism of action of this intervention is developed. This study will determine the mechanism underlying the effects of DBS of STN and GPi by examining in the MPTP monkey model of PD: 1) the effect of stimulation in the STN and GPi on neuronal activity and on neurotransmitter release in different portions of the basal ganglia-thalamocortical circuit, 2) the role of GPe in mediating the effect of stimulation in the STN and GPI, in mediating the development of parkinsonian motor signs and as an alternative site for stimulation for the treatment of PD and 3) determine the effect of stimulation in the STN and GPI on cortical function. The experiments will use a combination of single cell recording, microdialysis, and 18F-fluoro-deoxy-glucose

Principal Investigator: WALKER, PAUL D
Grant Number: 5R01NS039013-04
Title: SEROTONIN CONTROL MECHANISMS OF BASAL GANGLIA FUNCTION

Abstract: Attempts to develop new and effective treatments for movement disorders such as Parkinson's disease have been hampered by an insufficient knowledge of how basal ganglia receptor systems adapt to the consequences of dopamine depletion. This research focuses on determining the role of upregulated serotonin 2A receptors, which we hypothesize provide a mechanism for serotonin to exert greater control over basal ganglia transmission and locomotor function under conditions of dopamine depletion. Our preliminary studies indicate that the target of the serotonin 2A receptor mechanism is the DIRECT striatonigral pathway which utilizes tachykinin neuropeptides colocalized with GABA. New experiments of this application will test the central hypothesis that: upregulated serotonin 2A receptor signaling provides a mechanism for serotonin to enhance striatonigral transmission under conditions of dopamine depletion which influences basal ganglia function and animal behavior. In Specific Aim 1, we will determine the functional consequences of an upregulated serotonin 2A receptor system on serotonin signal transduction within the dopamine depleted striatum by measuring serotonin 2A receptor binding, its linkage to phosphoinositol hydrolysis, its modulation of striatal membrane excitability, and its ability to trans-synaptically regulate striatal tachykinin and GABA expression. In Specific Aim 2, we will determine if tachykinin striatonigral neurons react to the stimulation of upregulated serotonin 2A receptors in the dopamine depleted animal by increasing tachykinin and GABA transmission in the substantia nigra. We will also study the impact of this regulation on locomotor behavior. Finally, in Specific Aim 3, we will determine how an upregulated serotonin 2A receptor system influences the ability of the striatonigral system to regulate basal ganglia dopamine and GABA metabolism, and how these systems influence behavioral recovery of the dopamine depleted animal. Information obtained from these studies will contribute to a better understanding of basal ganglia function and may change how serotonin pathways are considered when designing new pharmacological strategies for diseases which affect dopamine transmission. -

Principal Investigator: Walters, Judith
Grant Number: 5Z01NS002139-30
Title: Pharmacology And Physiology Of The Substantia Nigra And Basal Ganglia

Abstract: Unavailable

Principal Investigator: WICHMANN, THOMAS N
Grant Number: 5R01NS042250-04
Title: Basal ganglia discharge patterns in parkinsonism

Abstract: The basal ganglia are part of larger circuit that involves thalamus and cortex. Cortical inputs reach striatum and subthalamic nucleus (STN), and are transmitted via internal pallidal segment (GPi) and substantia nigra pars reticulata (SNr) to influence the activity of thalamocortical neurons. The function of this circuitry is disturbed in Parkinson's disease because of loss of dopamine in the basal ganglia. Besides changes in discharge rates, basal ganglia neurons also develop significant abnormalities in their discharge patterns in parkinsonism. One of the most salient abnormalities is the appearance of synchronized oscillatory discharge in STN, the external pallidum (GPe), GPi/SNr, and frontal cortex (detected by EEG). Available data suggest that this may result from altered activity along the cortex-STN-GPi/SNrthalamocortical route. With a combination of extracellular basal ganglia recordings and EEG, the proposed primate experiments explore the relationship between oscillatory activity in cortex and basal ganglia and will test the hypothesis that oscillatory discharge in the cortex-basal ganglia circuitry contributes to parkinsonism. The correlation studies under specific aim (S.A.) 1 assess the link between neuronal discharge in the basal ganglia (GPe, STN GPi, SNr) and EEG with simultaneous recordings in both brain regions. The importance of striatal or extrastriatal dopamine loss for the development of oscillatory discharge in parkinsonism will be tested under S.A. 2 by studying changes in oscillatory activity in basal ganglia and cortex induced by microinjections of the dopamine receptor agonist apomorphine at striatal and extrastriatal basal ganglia sites in parkinsonian animals. The experiments under S.A. 3 will test whether blockade of glutamate receptors in STN (blocking corticosubthalamic inputs) reduces oscillatory activity in basal ganglia and cortex. Finally (S.A. 4), the hypothesis will be tested that synchronized oscillatory discharge in the basal ganglia, induced by electrical stimulation of STN with bursts of stimulation pulses at burst rates between 2 and 30 Hz, disrupts motor performance and induces parkinsonian motor abnormalities in normal monkeys. These studies will help to understand the significance of oscillatory discharge in the basal ganglia and cortex in parkinsonism. This may provide guidance in the development of drug treatments directed at normalizing abnormal discharge patterns, and may help to understand the mechanism of action of existing treatments for Parkinson's disease, including dopamine receptor agonists, glutamate receptor antagonists, and deep brain stimulators. -

Principal Investigator: WICHMANN, THOMAS N
Grant Number: 5R01NS040432-04
Title: Influence of subthalamic nucleus on striatal dopamine

Abstract: Degeneration of the dopaminergic nigrostriatal tract results in Parkinson's disease. Over the last years, rodent studies have provided evidence that the activity of the source neurons of the nigrostriatal tract in the substantia nigra pars compacta (SNc) is modulated by afferents from the subthalamic nucleus (STN). Increased STN output, a central feature of most models of parkinsonian pathophysiology, could impact SNc function in early parkinsonism, helping to compensate for the loss of striatal dopamine by increased driving of nigrostriatal neurons. In rodents, STN and SNc are linked via excitatory glutamatergic projections, or via inhibitory pathways involving GABAergic neurons in the substantia nigra pars reticulata (SNr). Activation of the excitatory projections results in increased bursting in SNc, whereas activation of the inhibitory projections lowers the average discharge rates in SNc. Our preliminary data in primates have also demonstrated excitatory and inhibitory effects of STN stimulation on SNc activity, and have indicated that striatal DA levels may be increased with STN stimulation and reduced with STN inactivation. Effects on striatal dopamine may be explained by the direct synaptic STN-SNc interaction, by actions mediated via long loop circuits through thalamus and cortex, as well as by presynaptic mechanisms. The proposed experiments will explore the STN-SNc relationship in primates, with the general hypothesis that STN activation will result in increased burst discharges in SNc and increased dopamine levels in the striatum, while STN inactivation will result in the opposite. A combination of electrophysiologic, microdialysis and anatomic methods will be used to assess effects of transient manipulations of STN activity, induced by intra-STN injections of the GABA receptor agonist muscimol or the GABA receptor antagonist bicuculline, on the neuronal activity in SNc and SNr and on striatal dopainine levels (S.A. V 1/2). Similarly, effects of "deep brain" stimulation and lesions of STN will be studied to assess the impact of these commonly used neurosurgical interventions on SNc and SNr activity, and on striatal DA. In the case of STN lesions, the density of glutamate and GABA receptors in SNc will also be determined (immunoautoradiography) as an inverse measure of the strength of glutamatergic and GABAergic inputs to SNc. These studies will provide insight into the role of the STN-SNc interaction under normal and parkinsonian conditions and will help to understand the mechanisms of action of neurosurgical treatments aimed at SIN in parkinsonian patients. -

Principal Investigator: YEN, SHU-HUI C
Grant Number: 1R01NS048052-01A1
Title: Modeling Neurofibrillary Degeneration

Abstract: Progressive supranuclear palsy shares its defining pathologic signature, neurofibrillary tangles (NFT) consisting primarily of hyperphosphorylated tau, with numerous neurological diseases, including Alzheimer's disease, corticobasal degeneration, Pick's disease as well as frontotemporal dementia and Parkinsonism linked to chromosome 17. To improve our understanding of the mechanism underlying NFT formation and its functional impacts we have developed cellular models that produce tau filaments with morphological and biochemical characteristics of human tauopathies. The models consist of conditional transfectants generated from human neuroglioma [H4] and neuronal [BE(2)-M17D] cells in which transgenic production of wild-type or mutant tau is regulated via the TetOff inducible mechanism. Preliminary studies demonstrated that treatment of these cells with 4-hydroxynonneal (HNE), proteosomal or calpain inhibitors enhances the assembly of disulfide-linked tau aggregates. The results suggest that cellular insults such as oxidative stress and deregulation of proteases may play a role in the formation of NFT. We will employ this cellular model to uncover the molecular mechanism underlying tau aggregation induced by various insults. The Specific Aims of our proposal are: (1). To test if factors implicated in the etiology and pathogenesis of human tauopathies exacerbate tau aggregation in conditional transfectants, (2). To determine if the enhanced aggregation is associated with changes in tau solubility/partition, phosphorylation, degradation and oligomerization, (3). To investigate whether such exacerbated tau aggregation is associated with altered level or state of activation/activity of particular kinases, proteases and/or proteasomes, and (4). To study if progression of the exacerbated assembly of tau aggregates can be blocked through deregulating kinases/proteases. The results are likely to provide valuable information for a rational design of therapeutics to treat neurofibrillary degeneration. -

Principal Investigator: YOUNG, ANNE B
Grant Number: 3P50NS038372-05S2
Title: MGH/MIT PARKINSONS DISEASE RESEARCH CENTER

Abstract: Unavailable

Principal Investigator: YOUNG, ANNE B
Grant Number: 2P50NS038372-06A1
Title: MGH/MIT MORRIS UDALL CENTER OF EXCELLENCE IN PD RESEARCH

Abstract: The MGH/MIT Morris Udall Center of Excellence in PD Research is taking a broad, collaborative and interactive approach to the study of Parkinson's disease. The Projects address critical questions concerning the selective vulnerability of dopamine neurons, the mechanism and consequences of Lewy body formation and alpha-synuclein aggregation, the neural systems consequences of parkinsonism and synuclein pathology, and molecular approaches for modifying this pathology. These issues will be explored using a range of systems, from yeast genetics, to mammalian cell culture, to rodent models to human postmortem material. The Center incorporates state-of-the-art technologies including high throughput yeast genetic screens to identify modifiers of synuclein aggregation and toxicity, viral vector gene transfer to study factors in mammalian cell culture and rodent models, multi-unit tetrode recordings to study striatal plasticity, fluorescence lifetime imaging to study protein-protein interactions, and laser capture microdissection and gene arrays to study transcriptional dysregulation. The Center has a Clinical and Training Core that provides care to patients with Parkinson's disease, gathers data on clinical features of the disease and response to therapy, solicits brain donations for neuropathological study, and trains outstanding clinician scientists to be future leaders in the field. The Center also has a Bioinformatics Core that serves to integrate and analyze data across the projects, and facilitate sharing of the information. The Administrative Core is charged with management of the Center and facilitating the sharing of information, ideas, and reagents among the investigators and with other components of the Udall Centers consortium. The investigators of the MGH/MIT Center are dedicated to a program of collaborative and interactive studies which will lead to better treatments for people with Parkinson's disease.-

Principal Investigator: ZASSENHAUS, HANS P
Grant Number: 5R01NS041785-04
Title: Pore opening: A target for mitochondrial DNA mutations

Abstract: Mitochondrial dysfunction is seen not only in late-onset neurodegenerative disease, such as Alzheimer's, Parkinson's, and Huntington's, but with aging in the normal brain as well. Since the frequency of mitochondrial DNA (mtDNA) mutations in the brain climbs hundreds to thousands of fold with age, it is widely thought that such mutations may contribute to cause mitochondrial dysfunction. To experimentally probe their pathophysiology, transgenic mice were constructed that rapidly accumulate specifically mtDNA mutations in cardiomyocytes. These mice reveal that mtDNA mutations - at frequencies commonly seen with age or disease in humans - indeed cause pathology. Characterization of mitochondria from those mice suggests a novel molecular mechanism for the pathogenesis of elevated levels of mtDNA mutations. As mutations rise so do the levels of mutant proteins encoded by the mitochondrial genome. Some of these mutant proteins will misfold. One of the major chaperones catalyzing protein folding in mitochondria is cyclophilin D (CyP-D), a peptidyl-prolyl cis/trans isomerase that also functions to regulate mitochondrial pore transition. Elevated levels of misfolded mitochondrial-encoded proteins are proposed to lead to dysfunction of CyP-D and, in turn, to dysregulation of pore transition. Catastrophic pore transition is known to cause massive disruption of calcium homeostasis in neurons and to signal cell death by apoptosis. To test these hypotheses, we propose to: 1) characterize the structural and functional alteration in CyP-D that occur when the levels of mtDNA mutations rise, 2) determine the basis for the alteration in mitochondrial pore transition that occurs when mutation levels rise, and 3) generate transgenic mice with an accelerated accumulation of mtDNA mutations in the brain to characterize the effect(s) of these mutations on the function of CyP-D and the permeability transition pore in neurons. These studies are broadly significant to understand molecular mechanisms for the pathogenesis of mtDNA mutations. Since such mutations may be an important contributing factor for many adult-onset diseases, these studies may provide insights into novel therapeutic strategies. -